BACTERIOLOGICAL PROFILE

OF BURN WOUND INFECTIONS IN A TERTIARY CARE CENTRE AND CONFIRMATION BY QUANTITATIVE BACTERIAL ASSAY

Dissertation Submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment of the regulations

For the award of the degree of

M.D. (MICROBIOLOGY)

BRANCH – IV

APRIL 2015

THANJAVUR MEDICAL COLLEGE, THANJAVUR

THE TAMIL NADU Dr. MGR MEDICAL UNIVERSITY,

CHENNAI, TAMIL NADU.

CERTIFICATE

This is to certify that the dissertation entitled "**BACTERIOLOGICAL PROFILE OF BURN WOUND INFECTIONS IN A TERTIARY CARE CENTRE AND CONFIRMATION BY QUANTITATIVE BACTERIAL ASSAY**" submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by Dr.R.Subbulakshmi at the Department of Microbiology, Thanjavur Medical College and Hospital, Thanjavur during the period from June 2013 to July 2014 under my guidance and supervision and the conclusions reached in this study are her own.

The Dean,

Thanjavur Medical College,
Thanjavur

Dr. P.Sankar,M.D.

Head of the Department,
Department of Microbiology,
Thanjavur Medical College,
Thanjavur

CERTIFICATE OF THE GUIDE

This is to certify that this Dissertation entitled "**BACTERIOLOGICAL PROFILE OF BURN WOUND INFECTIONS IN A TERTIARY CARE CENTRE AND CONFIRMATION BY QUANTITATIVE BACTERIAL ASSAY"** is the original and bonafide work done by Dr. R. Subbulakshmi under my guidance and supervision at the Thanjavur Medical College and Hospital, Thanjavur during the tenure of her course in M.D,(Microbiology) from May 2012 to April 2015 held under the regulations of Tamilnadu Dr. M.G.R. University, Chennai – 600032.

Place: Thanjavur

Head of the Department,
Department of Microbiology,
Thanjavur Medical College,
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DECLARATION

I, Dr. R. Subbulakshmi truly declare that the dissertation entitled "**BACTERIOLOGICAL PROFILE OF BURN WOUND INFECTIONS IN A TERTIARY CARE CENTRE AND CONFIRMATION BY QUANTITATIVE BACTERIAL ASSAY**" submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by me at the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period of June 2013 to July 2014. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place: Thanjavur (Dr.R.SUBBULAKSHMI)

Date:

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA-613 001

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submitted by Dr. R. SUBBULAKSHMI **of**

Dept. of MICROBIOLOGY **Thanjavur Medical College, Thanjavur**

was approved by the Ethical Committee.

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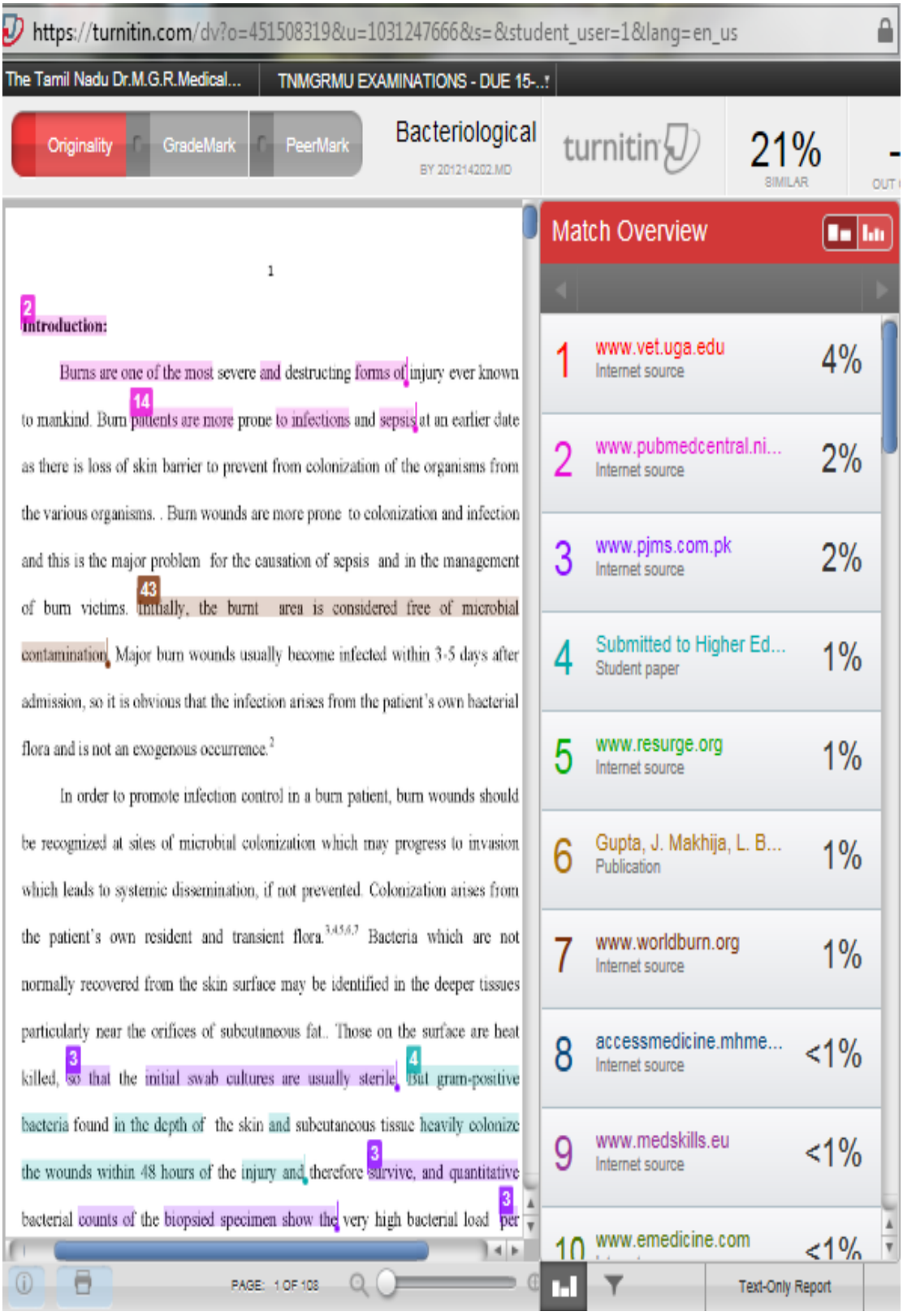
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Introduction:
Burns are one of the most severe and destructing forms of injury ever known to mankind. Burn patients are more prone to infections and sepsis at an earlier date as there is loss of skin barrier to prevent from colonization of the organisms from the various organisms. . Burn wounds are more prone to colonization and infection and this is the major problem for the causation of sepsis and in the management of burn victims. Initially, the burnt area is considered free of microbial contamination. Major burn wounds usually become infected within 3-5 days after admission, so it is obvious that the infection arises from the patient's own bacterial flora and is not an exogenous occurrence.[2]
In order to promote infection control in a burn patient, burn wounds should be recognized at sites of microbial colonization which may progress to invasion which leads to systemic dissemination, if not prevented. Colonization arises from the patient's own resident and transient flora.[3,4,5,6,7] Bacteria which are not normally recovered from the skin surface may be identified in the deeper tissues particularly near the orifices of subcutaneous fat.. Those on the surface are beat killed, so that the initial swab cultures are usually sterile, but gram-positive bacteria found in the depth of the skin and subcutaneous tissue heavily colonize the wounds within 48 hours of the injury and therefore survive, and quantitative bacterial counts of the biopsied specimen show the very high bacterial load per
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DEDICATED TO

My Eternal guide Lord Venkateswara

And three great men in my life

My Husband Raju, Brothers Sara and Senty

Without them I am not me today.

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I am very thankful to our honourable Dean, **Dr. K. Mahadevan, M.S**, Thanjavur Medical College Hospital, Thanjavur for permitting me to carry out this study.

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I express my sincere thanks to **Prof. Dr. G. Ravikumar, M.S., MCh., DNB (Gen), DNB (PLS);MRCS (Ed.),** Professor and Head of the Department, Plastic Surgery, TMCH, for his innovative ideas and valuable guidance throughout this study.

I am very fortunate to have our honored teacher **Dr. Eunice Swarna Jacob, M.D.,** Associate Professor, Department of Microbiology, Thanjavur Medical College and I express my heartfelt thanks for her valuable guidance, constant support given to me throughout this study.

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encouraged me to select this topic and giving their valuable suggestions towards the completion of this work.

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I would like to thank all my Co-PGs for their kind support and Co-operation. I am very thankful to all Laboratory Technicians for their help extended by them.

I wish to extend my thanks to all my family members especially to my beloved sons and my mother for their sacrifices and constant support during the entire period of this study.

I also extend my thanks to all the patients who participated in my study.

LIST OF ABBREVIATIONS

CFU …….. Colony forming units

MRSA ……. Methicillin resistant Staphylococcus aureus

VRSA ……. Vancomycin resistant Staphylococcus aureus

ESBL …….. Extended beta lactamases

MBL …….. Metallo-beta lactamases

MODS ……. Multiple organ Dysfunction Syndrome

SIRS ……... Systemic Inflammatory Response Syndrome

TBSA ……. Total Body Surface Area Burns

CoNS ……. Coagulase Negative Staphylococcus

WHO …….. World Health Organization

DALY ……. Disability adjusted life years

PAS …… Periodic Acid Schiff

GMS ……... Gomoroi'sMethenamine Silver

PPPBI ……. Programme for prevention of burn injuries

ICU ……. Intensive care unit

NPPBI …….. National Programme for prevention of Burn injuries

USAISR …… United States Army Institute of Surgical Research

INCRB ……. Indian National Crime Records Bureau

CLSI …….. Clinical and Laboratory Standards Institute

MDRO ……. Multi drug resistant organism

EDTA ……... Ethylene diamine tetra acetic acid

MHA ……... Mueller Hinton agar

IMP-EDTA … Imipenem –ethylenediamine tetra acetic acid

SD …… Standard Deviation

SEM …… Standard error of mean

SSG ……... Split skin graft

CDC …… Centre for disease control and prevention

UTI …… Urinary Tract Infection

E.coli …….. Escherichia coli

MIC …… Minimum inhibitory concentration

TSI …… Triple sugar iron agar

MR test …….. Methyl red test

VP test …….. VogesProskauer test

OF test ……. Oxidative Fermentative test

NR test …… Nitrate Reduction test

H2S …….. Hydrogen Sulphide test

ATCC ……... American type culture collection

NG …….. No growth

Pip/Tazo ….. PiperacillinTazobactum

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BACTERIOLOGICAL PROFILE OF BURN WOUND INFECTIONS IN A TERTIARY CARE CENTRE AND CONFIRMATION BY QUANTITATIVE BACTERIOLOGICAL ASSAY

Aim and Objectives:

Wound sepsis seems to be the most important and life threatening complications of all burned patients. Evaluation of the burns wound by a surface swabbing has been the principle method in my institution till date. This study emphasizes a method of quantitative bacterial count by a tissue biopsy technique for a more accurate method of assessing the burn wounds. Surface swabbing gives surface colonization,while a count $>10^5$ CFU/gm of tissue in the quantitative assay were likely to develop sepsis and also to predict graft bed receptiveness and safety of wound closure.

Materials and methods:

83surface swabs and 209 tissue biopsy specimens were collected from a total number of 83 patients. Biopsy was taken with a help of No.11 blade and Alley"s forceps. Then it was weighed and homogenized in a Lab Blender Stomacher 80. Four fold dilutions of the homogenate was made. Then they were inoculated in MacConkey and Blood agar plates and after 24 hours incubation colonies counted by formula.

Results and Observations:

Out of 83 patients, 54.22% were females and 45.78% were males.The most common age group affected was 16-30 years (51.81%)and 31.33% acquired 10-20% TBSA burns.61.45% were surface swab culture positive and 95.22% were tissue biopsy culture positive. More number of CoNS(14.13%), Micrococci(7.61%), Diphtheroids(2.17%)and culture negatives(15.22%) were reported in swab cultures but not in tissue cultures.Staphylococcus was the predominant organism isolated.Bacterial counts for the organisms ranged from 0.54 to 4.40 x 10^5 per gram of tissue. Bacterial counts < 10^5 yielded a graft take of 95-100%, while counts >10^5 yielded a graft take of 60%.

Conclusion:

Superficial swabbing is a simple, inexpensive and convenient method, but does not reflect the exact pathogen status,while quantitative bacteriology is also simple by the above method and provides an exact count of bacteria and provides most reliable data in predicting burn wound sepsis. This should be employed in every public and private burn care units to reduce burn related deaths.

Key words: Surface swabbing, tissue biopsy culture, quantitative assay, homogenization.

INTRODUCTION

INTRODUCTION

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Infection causes 50% to 60% of deaths in burn patients in spite of intensive therapy with antibiotics both topical as well as intravenous[1]. Burn wounds are highly susceptible to colonization and infection and this is the major problem in the management of burn victims. Initially, the burnt area is considered free of microbial contamination. Major burn wounds usually become infected within 3-5 days after admission, so it is obvious that the infection arises from the patient's own bacterial flora and is not an exogenous occurrence.[2]

In order to promote infection control in a burn patient, burn wounds should be recognized at sites of microbial colonization which may progress to invasion which leads to systemic dissemination, if not prevented. Colonization arises from the patient's own resident and transient flora.[3,4,5,6,7] Bacteria which are not normally recovered from the skin surface and sweat glands may be identified in the hair follicles particularly near the orifices of sebaceous glands. Those on the surface are heat killed, so that the initial swab cultures are usually sterile. But gram-positive bacteria found in the depth of sweat glands and hair follicles heavily colonize the wounds within 48 hours of the injury and

therefore survive, and quantitative counts of the biopsied specimen show the same 10^3 bacteria per gram of tissue, as found in the tissue prior to burning[8]. As the bacteria proliferates following burn injury and reach levels of greater than 10^5 bacteria per gram of tissue, they will break out of the hair follicles and glands and begin to migrate through the tissue, colonizing along the dermal sub-dermal interface[9].Perivascular growth is accompanied by thrombosis of vessels, necrosis of any remaining dermal elements, converting partial thickness burn to full thickness burn injuries.[10]

The subeschar tissue is the site where bacterial proliferation takes place. When the level of bacterial growth exceeds 10^6 or 10^7 , then microbial invasion into the bloodstream occurs. This is the most important cause, leading to septicaemia.[11]

Multiple studies from the United States Army Institute of Surgical Research (USAISR) made invasive burn sepsis synonymous with a bacteria count of 10^5 or greater than10^5 bacteria per gram of tissue. So monitoring the bacterial load has become an important tool in predicting and preventing invasive burn wound sepsis.[12,13,14]

Various modalities have been employed in monitoring sepsis in burn patients. It has been observed that a rapid Gram stain can reliably predict a microbial load of 10^5 CFU/gm of tissue if a single microorganism has been seen on a slide preparation[15,16]. Some studies

were done prioritizing histopathology over all other methods as it demonstrates true invasion.[17] This histopathological evalution can detect bacteria, fungus by Periodic acid-Schiff (PAS) and Gomori's methenamine silver (GMS), although cultures must be obtained to definitively identify and give sensitivity of the pathogen. Another technique which has been utilized is the velvet pad surface imprint technique. Dermabrasion as a sampling method has also been used.[18] Wound fluid sampling can also be done when copious volume of burn wound fluid exists, sampling by needle aspiration is employed. This is superior to swabbing and it requires strict aseptic precautions so that samples can be retrieved without any significant exogenous contamination.[18]An alginate-tipped swab can be used to perform a full quantitative analysis, since the swab will dissolve and release all associated microorganisms when transferred to an appropriate diluent. Despite its widespread use, there is debate over the value of the swab sampling technique and the value of cleansing a wound before swabbing is performed. A variety of other techniques, including the dry and presoaked velvet pad, filter paper disks and cylinder scrubbing, have also been used to sample superficial wound fluid for microbiological analysis. The immense number of sampling methods available creates a problem since all are reputed to have benefits and there is no single, universally accepted method. Thus the debate and controversy continues regarding

the type of sample required to provide the most meaningful data.[19,20,21] The three most popular methods are surface swabbing by cotton tipped swabs, blood cultures and quantitative bacterial assay by deep tissue biopsy techniques. The value of superficial cultures in wound assessment has been questioned and it is stated that purulent wound fluid may fail to yield growth whereas biopsied tissue may yield significant numbers of bacteria.[22] Swab sampling has been challenged on the basis that the superficial colonizers do not reflect the organisms of deeper tissue and that subsequent cultures do not correlate with the presence of pathogenic bacteria.[21] But the procedure of superficial swab samples is simple, inexpensive, non-invasive and convenient for the majority of wounds. On the other hand, blood cultures yield a positive culture report only at a later date even when the organisms are present . Tissue biopsy is the gold standard for microbial conclusive evidence. This method is most beneficial in determining the optimal time for skin grafting and surgical wound closure.[23] However, the value of a single biopsy specimen particularly in slow healing chronic wounds is debatable. So multiple specimens are taken for a more accurate diagnosis.

Bacteriological assessment is done on:

- **The day of admission,** as it is important to know the bacteriological status at the commencement of treatment.

- **On the third or fourth day**, when the period of maximum exudation is over to assess the bacterial colonization
- **Twice weekly** as there is an indication, according to clinical signs
- **Three days before any proposed grafting procedure**. So that the results are available before making a final decision about the surgery.[24]

So the main aim for any strategy dealing with burn wounds should have definite goals in achieving an effective infection control .The goals should be to prevent transmission of exogenous organisms to patients or personnel, to control the transmission of endogenous organism, that is, the normal flora to sites at increased risk of infection and to protect and support existing defenses in patients with seriously impaired resistance. This study focusses on aspects of history, pathophysiology, microbial etiology and microbial analysis of burn wound infection with special emphasize laid on the surveillance of burn wound infections and its culturing techniques. This study is also aimed at providing preventive measures of burn wound infections and reviewed in the context of infection control by a combination of many procedures such as aseptic precautions, surgical intervention at appropriate time, culturing methods, therapeutic strategies, environmental control and strict adherence to recommended policies and procedures by the entire burn care team.

Surface wound swabs are currently used in many centres in India do not give the exact count of pathogens involved in burn wound sepsis; they throw light on results at times.[25,26] Therefore in order to monitor burn wound, quantitative bacteriology is essential and there should be a technique for monitoring bacterial counts. Wound sepsis seems to be the most important and life threatening complications of all burned patients. Evaluation of the burns wound by a surface swabbing has been the principle method in my institution till date. This study emphasizes a method of quantitative bacterial count by a tissue biopsy technique for a more accurate method of assessing the burn wounds. Surface swabbing gives surface colonization and may not accurately reflect the organism causing wound infection while a count of 10^5 CFU/gm of tissue in the quantitative assay were likely to develop sepsis and also to predict graft bed receptiveness and safety of wound closure

AIM AND OBJECTIVES

AIM

To emphasize a method of quantitative bacterial assay by tissue biopsy technique for accurate microbial assessment of burn wound infections.

OBJECTIVES

1. To study the bacteriological profile of burn wound infections in Thanjavur Medical College Hospital.
2. To do Quantitative Bacterial Assay in burn wound infections
3. To study the antimicrobial susceptibility pattern of the organisms isolated.
4. To study the resistance pattern of the organisms isolated from the burn wounds.
5. To emphasize the importance of quantitative bacterial assay to provide clinical guidelines for reconstructive procedures.
6. To assess the clinical outcome of patients who underwent reconstructive procedures following guidance of quantitative bacterial assay.
7. To predict sepsis by this method and thus aiding its prevention.

REVIEW OF LITERATURE

HISTORICAL REVIEW

Since the discovery of the first flame, the exothermic combustion reaction of oxygen and carbon have exposed human flesh to significant destruction and disfigurement in the form of burns.[27] The history of the treatment of burn injuries and burn care has slowly evolved as a rational treatment process from ages unknown. Table 1 shows the history of treatment of burns since man had evolved.[28]

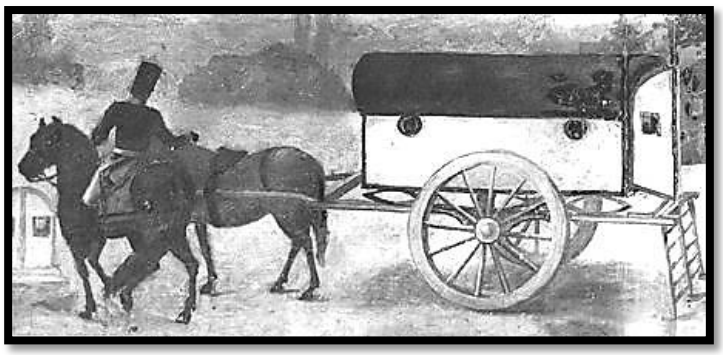
Table 1 - Burn treatment history

Neanderthal man	Extracts of plants
Smith papyrus (1500 BC) Egyptians	Gum and goat's milk mixed with mother's milk, strips soaked in oil
Chinese (600-500 BC)	Extracts of tea leaves
Hippocrates (430 BC)	Swines semen, resin, and bitumen Oak bark solutions
Celsus (ancient Rome)	Honey and bran
Galen (ancient Rome)	Vinegar and wine
Rhases (9th century)	Cold water
Pare (1517 – 1596)	Excision and ointments
David Cleghorn (1792)	Vinegar and chalk poultice
Edward Kentish (1797)	Pressure dressings
Syme (1827)	Wool dressings
Lisfranc (1835)	Calcium chloride dressings
Passavant (1858)	Saline dressings
Tomasalis (1897)	Salt water injections

From Salisbury RE:Thermal burns. In McCarthy JM, ed:Plastic Surgery, Philadelphia, WB Saunders, 1990:788

DR.AMBROSE PARE

In 16th century ***Ambrose Pare***, a French Surgeon practiced burn wound care for the war victims. He was called as the ***Father of modern Surgery***. He used invasive emollient Salves and compression dressings for burn wounds.[29] He also altered the discipline of Surgery and approach to burn wounds. In 18th century ***John Hunter*** a Scottish Surgeon worked on Burns Surgery[30]. In 19th century ***Dr. Jacob Bigelow*** an Harvard Physician practiced evidence based medicine in burn care. He conducted control studies in rabbits by adding a stimulant such as turpentine versus immersing burns in vat of ice water. His results were published in the first journal of *The New England Journal of Medicine, Surgery and Collateral Branches of Science*.[31] ***Dr. Dominique Jean Larry***, King Napoleon's chief Surgeon found out effective treatment of burns by a wax based paste known as cerate to cover burns. Also found ***"flying ambulance"*** consisting of 113 men



FLYING AMBULANCE

DR.DOMINIQUE LARRY'S FLYING AMBULANCE

with 12 light and 4 heavy transport carriages to carry burn patients to central medical port.[32]

Dr. Guillaume Dupuytren called as ***Father of modern Plastic Surgery***.[33,34] He was the first person to describe the pressure lines and termed them as ***"contractures"*** and also found treatment for it. He also showed that debridement of dead tissue and application of silver nitrate, emollients and cerate along with compression dressings improved burn wounds. The combination of all this reduced the chance of infection, promoted healing and improvement of rehabilitation.[35,36,37] Variation of these treatments remain in use even today, nearly 200 years after Dupuytren's time.

DR. DUPUYTREN

In 1906, ***George Thomas*** a Dermatologist in the College of Physician and Surgeon in New York, published the 1st handbook of treatment of burns.[38] In 1917, during World War II, ***Sulfa drugs and Penicillin*** were discovered which changed the approach to the treatment of burns totally.[39] Lieutenant Colonel ***AJ Hull*** developed and patented No.7 paraffin associated with aseptic technique like hand washing which he proved reduced exposure to infections.[39] In the 20th century,

LouisPasteur developed aseptic techniques which decreased mortality and morbidity in burn patients.[30] In 1875***, Joseph Lister*** used boric acid and carbolic acid for burn wounds to kill bacteria.[28]***Dr. Harold Gillies*** a surgeon in Aldershot Burn unit, UK laid autologous skin graft and did reconstructive surgeries in burn patients.[35] *Robert Aldrich*, used ***tripledye method***.[35] Gentian violet seals the wound and kills the Gram positive bacteria such as Staphylococcus aureus, one of the most common infectious agent to complicate burns.[35] In 1943, The US Army Institute for Surgical Research (USAISR),[40] situated in Fort Sam Houston, Texas was formed in which 300 physicians, surgeons and allied health professionls were committed to burn care.[40]***Dr. Summer Koch and Harvey Allen*** of Chicago used petroleum jelly dressings for burns. This method gained attention to all the burn fraternity units and came to be known as ***"Allen Koch"*** method of burn treatment.[35] In 1930 ***Frank Underhill*** , after analyzing a group of people burned in a theatre accident, documented articles saying that burn shock and death was due to fluid loss and not due to toxins which was a popular theory of that time.[28] In 1965, ***Moyer and associates*** recommended Ringer lactate solution[28] for the burned patients .In 1978, ***Charles Baxter*** modified this recommendations with the Parkland formula. With these advantages in the understanding of burn shock and vigorous fluid resuscitation, a dramatic improvement in early survival occurred.[28] In 1960s, The US

HISTORICAL REVIEW

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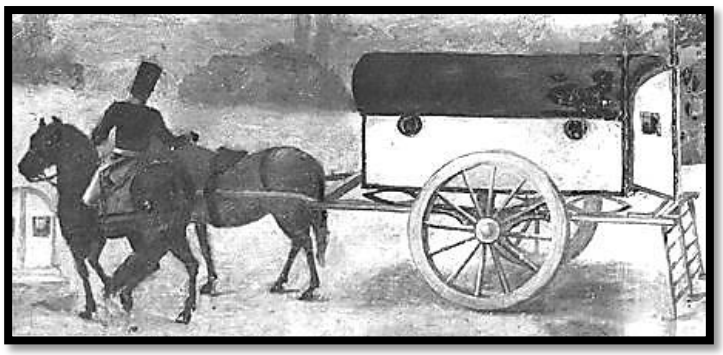

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Army Institute of Surgical Research team used ***The Magic Bullet***", the Penicillins which reduced infections and its complications and as a consequence death due to burns started to decline steadily.[35] In 1962, ***Dr.A Pruitt***, dramatically improved the burn surgeries and set research unit on burns and is maintaining it till date.[41] In 1963, the ***International Society for Burns Injuries*** was founded. In 1968, the ***American Burn Association*** **was founded**.[28]

Since 1970 , efficient management of burn wounds have been developed like **Collagen sheets** for I degree burns, **Fresh dried Human amniotic membranes**, **Nano sheets** and advanced surgery techniques like **Escharectomy** followed by **Split Skin grafting**. Monitoring of burn wound infections has also founded a drastic evolution. Over three decades ago, in 1974 **Loebl and colleagues** developed and evaluated a method for **quantitative bacterial cultures** of burn wound samples that have been widely adopted into practice even today by various modifications.

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well-developed and organized super- speciality. This global perspective about the outcome and quality of life for survivors of an often devastating injury affords us a special position in this field.

IMMUNOLOGY IN BURN INJURY

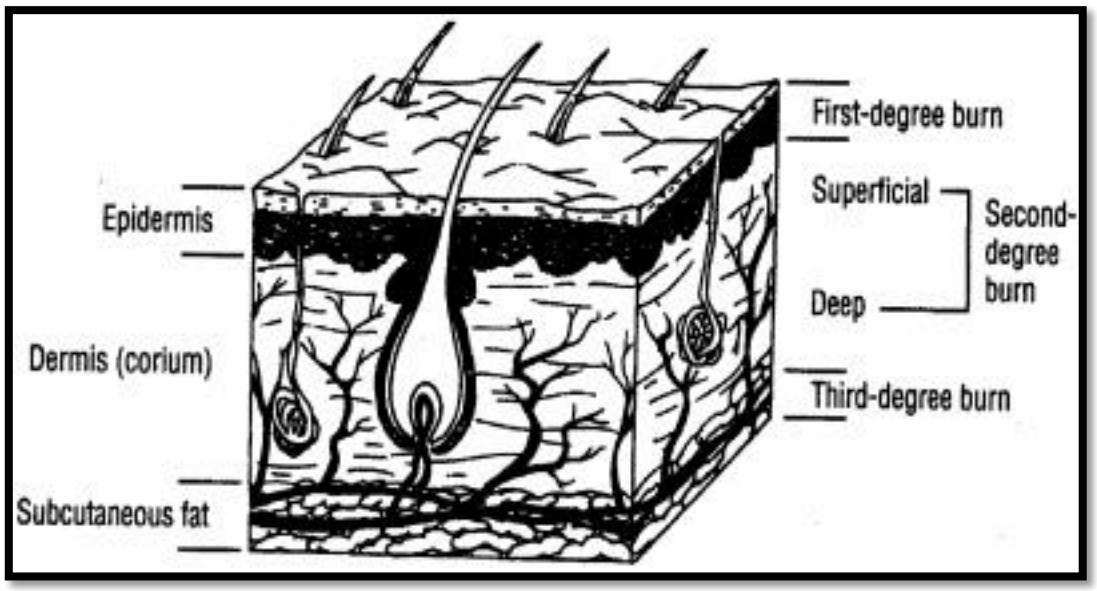
Severe burn injuries cause a state of immunosuppression that affects innate and adaptive immune responses. The substantial impact of immunocompromise on infection is due to effects on both the cellular and the humoral arms of the immune system.[42] Decrease in the number and activity of circulating helper T cells, increases in the number and activity of suppressor T cells, decrease in production and release of monocytes and macrophages, and diminution in levels of immunoglobulin follow major burns.[42] Neutrophil and complement functions also have been shown to be impaired after burns. The increased levels of multiple cytokines detected in burn patients are compatible with the widely held belief that the inflammatory response becomes dysregulated in these individuals; bacterial cell products play a potent role in inducing proinflammatory mediators that contribute to this uncontrolled systemic inflammatory response. Increased permeability of the gut wall to bacteria and their components (eg.,endotoxins) also contributes to immune dysregulation and sepsis.[42] Thus, a burn patient is predisposed to infection at remote sites as well as at the sites of burn injury. Another

contributor to secondary immunosuppression after burn injuries is the endocrine system; increasing levels of vasopressin, aldosterone, cortisol, glucagon, growth hormone, catecholamines, and other hormones that directly affect lymphocyte proliferation, secretion of proinflammatory cytokines, natural killer cell activity, and suppressor T cells are seen.[42]

PATHOPHYSIOLOGY OF WOUND INFECTION

The thickness of the epidermis and dermis vary with the site of the body, sex, age of patient, etc. Infants, young children, and elderly adults have a thinner dermal layer of their skin, resulting in deeper burn injury. The dermis has the ability to produce new epithelial cells to replace the cells that have been lost by the epidermis by means of shafts of appendages that are lined with epithelial cells. Both the skin layer and the connective tissue have nerve endings which provides a structural base for the skin renovation.[43] Immunologically, thermal injuries cause an immuno compromised state . This was evident as homografts are seen to survive well in these patients.[44,45] There are two types of immune responses, the innate and the adaptive responses. The first line of defense is the innate immune response immediately after the damage of skin by the burn wound.[46] When the wound is extensive, or involving major organ systems or is deep, the systemic component of immunity comes into play.

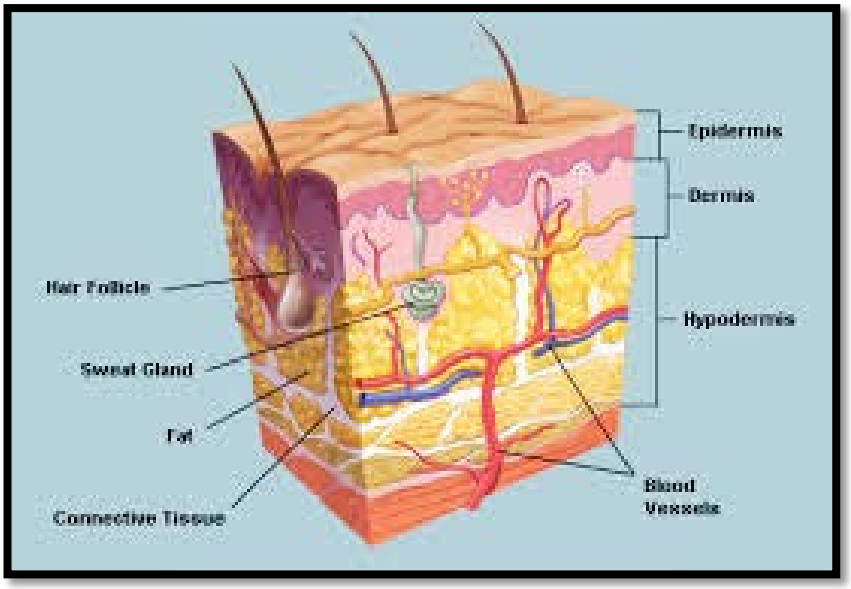
NORMAL SKIN



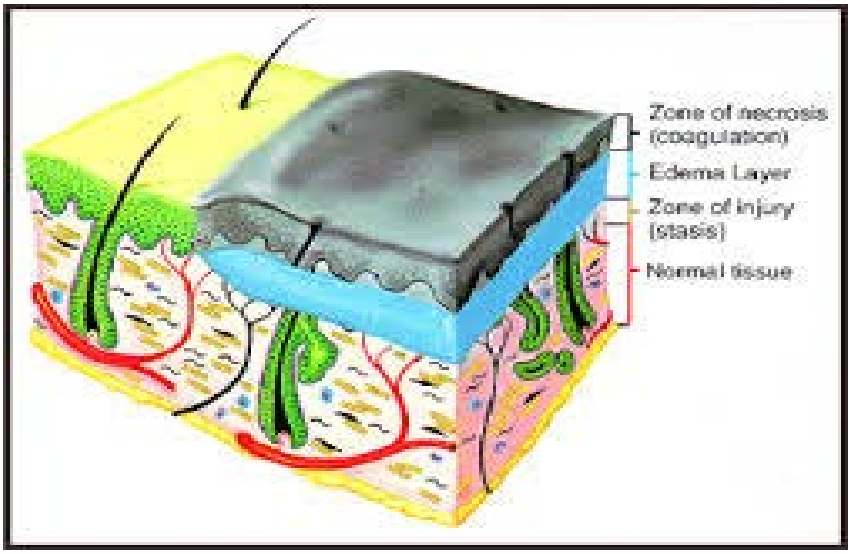
This response leads to varied spectrum of presentation which results in serious complications like systemic inflammatory response syndrome (SIRS), severe sepsis, and multiple organ dysfunction syndrome (MODS) which result in increased morbidity and mortality.[47] Immediately after a burn, the body tries to maintain homeostasis by a process of contraction, retraction, and coagulation of blood vessels. Three zones have been described within the wound:

- I **Zone of coagulation**, which comprises the dead tissues that form the burn eschar that is located at the centre of the wound.

NORMAL SKIN



ESCHAR AND SUBESCHAR TISSUE



- II **Zone of stasis**, which comprises tissues adjacent to the area of burn necrosis that is still viable but at risk for ongoing ischemic damage due to decreased perfusion.
- III **Zone of hyperemia**, which comprises normal skin with minimal cellular injury, that has predominant vasodilatation and increased blood flow as a response to injury.

BURN WOUND DEPTH

- **First Degree Burns:** Involves damage to only the epidermis and is rarely clinically significant but is very painful. The involved area is initially erythematous due to vasodilation. It is quickly followed by desquamation followed by complete scarless healing within 7 days.
- **Second degree burns:** By definition they are partial thickness burns and are divided into superficial and deep category.

1. **Superficial burns** involves destruction of whole of epidermis and with varying portions of dermis. These injuries are very painful. Blistering is often present. Healing occurs very rapidly and completely. Scarring is very minimal.
2. **Deep burns** involves destruction of most of dermis excluding the epithelial appendages. Heat kills the nerve endings rendering the

wound relatively insensate. Blistering is usually absent. Healing is very slow. Intense scarring is present.

- **Third degree burns:** Also called as full thickness burns and involves the entire thickness of burns .There is no chance of healing. So they are routinely treated with excision and skin grafting.

The burn wound surface is a protein-rich environment consisting of avascular necrotic tissue (eschar) that provides a favorable niche for microbial colonization and proliferation. The lack of vascularity leads to an impaired migration of host immune cells and a restriction in delivery of systemically administered antimicrobial agents, while toxic substances released by eschar tissue further impair the local host immune responses. Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms.[48,49,50,51,52] Loss of cutaneous barrier facilitates entry of the patient's own flora and of the organisms from the hospital environment into a burn wound. Initially, the wound is colonized with Gram-positive bacteria from the surrounding tissue, but the number of bacteria grows rapidly beneath the burn eschar, reaching **~$8.4x10^5$ CFU/g** on day 4 after the burn.[42] By day 7, the wound is colonized with other microbes, including gram-positive bacteria, gram-negative bacteria, and

yeasts derived from the gastrointestinal and upper respiratory flora. Invasive infections either localized or systemic, occurs when these bacteria penetrate viable tissue.[42] In the light of any difficulty in evaluating burn wounds solely on the basis of clinical observation and laboratory data, wound biopsies are necessary for the definitive diagnosis of infection. The timing of these biopsies can be guided by clinical changes, but in some centres burn wounds are biopsied routinely at regular intervals. The biopsy specimens is examined for histologic evidence of bacterial invasion, and quantitative microbiologic cultures are performed. The presence of $>10^5$ viable bacteria per gram of tissue is highly suggestive of invasive infection and of a dramatically increased risk of sepsis.

MICROBIAL ETIOLOGY

Microorganisms colonizing the burn wound originate endogenously from the patient's skin, gastrointestinal and upper respiratory tracts. There are abundant microflora normally present in the gut, oral cavity and vagina. Microorganisms maybe transferred to a patients skin surface by contact with contaminated external environment, water, fomites, air and also contaminated hands of the health care workers.[53] Colonization occurs in slow healing wounds which are continuously exposed to devitalized tissue.

Initial invaders are the Gram-positive bacteria coming either from the patient's endogenous skin flora or from the external environment.[54] Wounds with hypoxic environment are susceptible to colonization by various endogenous anaerobic bacteria. When wounds are investigated by appropriate microbiological techniques, anaerobes are found in large numbers. Wound colonization by yeasts and fungi occurs later due to the use of broad-spectrum antibiotic therapy.[55] Microorganisms acquired from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora.[56] Table 2 lists the microorganisms of the burn wound infections.[24,37]

Table 2-Microorganisms causing invasive burn wound infections

Gram positive	Staphylococcus aureus
	Methicillin Resistant Staphylococcus aureus
	Coagulase negative staphylococcus
	Enterococcus species
	Vancomycin resistant enterococcus
	Beta hemolytic Streptococcus Group C
	Beta-hemolytic Streptococcus Group A
Gram negative bacilli	Pseudomonas aeruginosa
	Escherchia coli
	Klebsiella pneumonia
	Serratia marcescens
	Proteus species
	Acinetobacter species
	Enterobacter species
	Citrobacter species
	Stenotrophomonas maltophila
	Providencia species
	Morganella species
Anaerobic organism	Clostridium species
	Peptostreptococcus species
	Bacteroides species
	Prevotella species
	Porphyromonas species
	Propionibacterium species
	Eubacterium species
	Fusobacterium species
	Veillonella species
Fungi	Candida species
	Aspergillus species
	Fusarium species
	Alternaria species
	Rhizopus species
	Mucor species
Viruses	Herpes simplex virus
	Cytomegalovirus
	Varicella zoster virus

Prior to the antibiotic era, Streptococcus pyogenes (Group A beta-hemolytic Streptococci) was the predominant pathogen implicated in burn wound infections and a major cause of death. With the introduction of Penicillin, in 1950's, Streptococci was wiped off and replaced by Staphylococcus aureus. Till today, Staphylococcus aureus remains a common cause of early burn wound infection. Slowly then, evolved among the Gram-negative organism was Pseudomonas aeruginosa which originated from the patient's gut flora or an environmental source and became one of the foremost causes of burn wound infections in many centres.[59] There was a steady increase in the incidence of rare pathogens also.[60] Less commonly, infections due to anaerobic bacteria typically occur secondary to electrical burns or open wound dressings were used instead of occlusive dressings.[61]

Development of antimicrobial resistance throughout the world against a variety of bacterial and fungal pathogens, particularly nosocomial isolates limits the therapeutic options available for an effective treatment.[59] Methicillin resistant Staphylococcus aureus, Vancomycin resistant Enterococcus and multi drug resistant Gram-negative bacteria that possess several types of beta-lactamases, Amp C beta lactamases and metallobeta-lactamases have been emerging as

serious pathogens in hospitalized patients.[58] Fungal pathogens, particularly Candida species, have increasingly become important opportunistic pathogens due to the use of broad-spectrum topical and systemic agents when infection occurs in the burned patient and have demonstrated increasing degrees of antifungal drug resistance.[62]

EPIDEMIOLOGY OF BURN INFECTIONS

The development of infection in a burn patient depends on the presence of three conditions, often called the chain of events:

(1) A source of organisms capable of producing disease

(2) A mode of transmission, and

(3) A susceptible host

SOURCES OF ORGANISMS

Sources of organisms can be found within the patient's own endogenous flora, including organisms commonly harboured on the skin in the gastrointestinal tract, and in the upper respiratory tract. The types, amounts, and relative antimicrobial resistance of these organisms vary depending upon the patient's age, percentage of the total body surface area (TBSA) burned[62] and the antibiotic and other therapies needed to treat the burn injury. 85% - 95% of infections in burn patients originated

from the patient's endogenous flora[63] . Exogenous organisms emanate from the surrounding inanimate environment and from personnel. These organisms are generally more resistant to antimicrobial agents than endogenous organisms[63,64] .

MODES OF TRANSMISSION

Modes of transmission include contact, droplet, and airborne spread. Among burn patients the primary mode of transmission is direct or indirect contact, principally via (1) the hands of personnel caring for the patients and (2) contact with inappropriately decontaminated equipment. Burn patients are unique in their susceptibility to colonization and infection from organisms in the environment and in their propensity for dispersing organisms into the surrounding environment, the degree of which is directly related to the percentage of burn injury. Adequate understanding and appreciation of this fact are essential to establishing effective infection control strategies for these patients.

HOST SUSCEPTIBILITY

The body has three principal defenses against infection: physical defences, nonspecific immune responses , and specific immune responses. Alterations of these defences determine the individual's susceptibility to infection. Physical defences are greatly altered by

consequences of burn injury. Intact skin provides a physical barrier, normal flora, low pH maintained by fatty acids and dryness, desiccation and desquamation which is altered altogether by the burn injury. This is evidenced by the loss of epidermis and all or part of dermis, depending on depth of injury. This allows penetration of organisms into viable tissue of wound and into body, including the bloodstream. Also fosters shift in colonizing flora from skin organisms (coagulase-positive and negative streptococci, diphtheroids) to enteric gram negative organisms, pseudomonas and fungi. Invasive devices contribute to colonization by hematogenous seeding of organisms from the burn wound[65] . Extremes of age associated with changes in the body's defence mechanisms both in immune system functioning and in physical mechanisms. In elderly patients, preexisting medical conditions and waning immune function are of particular consequence because both can increase these patient's susceptibility to infection[62]. In children, susceptibility to common communicable childhood diseases poses a challenge for infection control and this may cause serious consequences if superimposed in a patient with a burn injury[66] .

GLOBAL BURNS LOAD

Burn injuries are among the most devastating of all injuries and a major global health crisis.[67,68]Burns are the fourth most common type of trauma

worldwide, following traffic accidents, falls, and interpersonal violence.[69,70] Approximately 90 percent of burns occur in low to middle income countries, regions that generally lack the necessary infrastructure to reduce the incidence and severity of burns. According to Rethink Burns, ReSurge International ,International Medical Corps,2013 , half of the world's population, three billion people, still use open flames to cook for their families and to heat and light their homes. Ninety-five percent of all burns injuries happen in developing countries where prevention is almost non-existent and medical care for burns is extremely limited. This caused millions of needless deaths or disabilities, even for relatively minor burns. Without immediate access to adequate burn care, burn injuries are left to heal by themselves, creating scar tissue (contractures) that can destroy function and movement, and cause disfigurement in ways unimaginable. In the developing world, countless men, women and children suffer unnecessarily from injuries and disabilities that could be prevented and treated.[73]

Nearly 11 million people worldwide are burned severely enough to require medical attention annually, according to WHO estimate of 2004. More women worldwide are severely burned each year according to new 2010 Global Burden of Disease Study, burn remain a leading cause of morbidity and mortality.

According to the Global Burden of Disease Study,2010, more than 330,000 people die of burn injuries every year, 307,000 in developing countries; 43% are under 30 years; 20% are children; more than half live in South Asia (India, Bangladesh, Nepal, Pakistan, Bhutan and Afghanistan); and a more than a fourth live in Sub- Saharan Africa.

Burns also cause more than 19 million DALYs (Disability adjusted life years); productive year lost to being disabled from a disease or injury. It is also in the top 25 causes of morbidity in South Asia and Sub Saharan Africa; the 28th in all developing countries, 27th for women and girls.

South Asia is at the epicenter of the burn crisis, and nearly a third of all deaths from burns globally happen to women and girls in that region. There, more women and children die from severe burns than from any other disease. It is also the 6th leading cause of lost productive years (DALYs) for women aged 15-49.

In Sub Saharan Africa, burns are also the 19th leading cause of death, the 16th for the ages 15-49. Infants in Africa have 3 times the incidence of burn deaths than infants worldwide.

WHO mortality data that includes the International Classification of Disease Codes which allows disaggregation into subtypes of burns. These data show fire-related burns made up of 93% of all deaths, scalds

contributed 5.4% and the rest; 1.6% were as a result of contact, chemical or electrical burns[74] .

BURNS LOAD IN INDIA

Burns is a special type of trauma which is dependent on many factors like the habits of the community, the culture of the society, the socioeconomic status of the people, the religious beliefs, environmental conditions and the psychological behavior of the population.[75] All these factors feature in causation of burns in India. Fire is an integral part of Indian lifestyle. Fire is used in different forms during daily chores. Be it morning worship, cooking, lighting and festivities, everything revolves around fire. Hence it is likely that the Indian community is more prone to the occurrence of burns. Understandably, the burn is more prevalent in people of lower socioeconomic status.[75] Electricity is unsafe in many of our houses and establishments, cooking gas is prone to accidents, chemicals that can cause accidental burns are easily available, fire crackers being a part of all our festivities and many more issues favor the occurrence of burns in India. On the other hand, in the developed countries, individuals do not come in contact with fire very often. The closest they come to fire is while lighting candles for worshiping in the church. The presence of smoke detectors prevents use of fire at home, as well as at the workplace.[75]

Approximately 7 million people sustain burns in India every year, out of which 700,000 patients (10%) require admission[76] . Half of the hospital burn patients sucuumb to the injury. Majority of the burns in India do not report to hospitals. 70% of Indian population live in the villages and rural areas. They do not have access to hospitals. Almost all minor burns do not report to hospitals. Many major burns are treated in place other than recognized burn hospitals and quite a few by a unqualified persons and hence remain unreported[75] . Only a section of the patients with major and moderate burns report to hospitals for treatment and are reflected in the hospital statistics. The patients who report to the hospitals form only the tip of the iceberg.

In contrast to the developed world, the burn load in developing countries is many folds higher. As per the report of the Indian National Crime Records Bureau (INCRB) of 2007, out of 340,794 total accidental deaths, 20,772 (6.2%) were due to fire accidents. There were 10,391 (8.5%) deaths classified as suicidal burn deaths out of a total of 122,637 suicidal deaths. The mortality due to burn injuries was reported as 3.5 per 100,000 population.[77]

India, the second most populous country in the world over a billion people has an estimated annual burn incidence of 6-7 million, based on data from three major hospitals when extrapolated to whole of the

country. This is the second largest group of injuries after road accidents. But there is a silver lining that 90% of all burn injuries are preventable. This burn scenario is grave not only due to the high incidence but is also compounded by absence of any organized burn care at primary and secondary health care level. Patients have to travel a long distance to metropolitan cities for management of their burn injuries. The recent rise in the incidents of terrorist activities and other man-made disasters, are contributing to a quantum jump in Burn injury cases also highlights reason for national preparedness to cope with the challenge of this Public Health Programme.

So the Ministry of Health and Family Welfare, government of India have under consideration a proposal to introduce a National Programme for Prevention of Burn Injuries (NPPBI) in all the states/Union Territories of the country during the 12^{th} five Year Plan (2012-17).

The goal of National Programme for Prevention of Burn Injuries would be, as the name itself suggests, to ensure prevention of Burn Injuries, provide timely and adequate treatment in case burn injuries do occur, so as to reduce mortality, complications and ensuring disabilities, and to provide effective rehabilitative interventions if disability has set in. Having identified that burn injury is a potential Public Health Hazard, in

order to reduce incidence and its consequential trauma in the country, a coordinated programme for Burn Injuries was conceived as a new initiative during the year 2010 and a Pilot Programme for Prevention of Burn Injuries (PPPBI) was launched in 3 states namely Assam, Haryana and Himachal Pradesh in a limited scale. Under this programme the government of India provided financial support to the extent of Rs.2.00 crores for the Construction of 12 bedded (8 beds + 4 ICU beds) Burn Unit and Rs.98 lakhs for the purchase of essential equipments for the Burn Unit.[78]

MICROBIOLOGICAL ANALYSIS OF BURN WOUND INFECTIONS

Diagnosis of burn wound infection based on clinical signs and symptoms alone is difficult. Monitoring of the burn wound by regular sampling is essential. This is done either by a surface swabbing or tissue biopsy . Tissue biopsy and quantitative bacterial count of verification of microbial invasion into viable unburned tissue have been the "**gold standard** " to confirm invasive burn wound infection[57]. As it is a quite laborious and costly procedure, many burn centres have shifted to procure burn wound surface swabs for qualitative or semiquantitative culture for monitoring the burn wound infection.[79,80,81]Deirde Church et al discuss the various diagnostic microbiological approaches to diagnose a burn

wound infection. They also suggest the current recommendations for surveillance of burn wound infection.

Review of the literature of various burn wound studies comparing surveillance of burn wound by surface swabs and burn wound biopsy always provides conflicting results about the best approach. The different studies provides conflicting results due to the following reasons:

Burned patients do not have same type of injury so that the severity and extent of the burn injury vary greatly from patient to patient. Many studies were done using various sampling techniques and microbiological methods , and comparative studies were done before the advent of early excision therapy.[84,85,86] Steer and colleagues, have reported the recent largest studies and compared the results of biopsy cultures and surface swabs.[87,88] In their initial study qualitative and quantitative bacterial counts were compared. Although there was a significant correlation between the bacterial counts obtained by biopsy and swab, the counts obtained by one method were poorly predictive of the counts obtained by the other. Also, parallel cultures taken on multiple occasions showed a significant correlation between bacterial counts obtained from two biopsies or two swabs simultaneously, but there was wide variation in bacterial densities from the same burn wound at the same time. The study concluded that the use of quantitative assay in

burns is limited by the unreliability of a single surface swab or biopsy sample to represent the whole burn wound.

Steer and coworkers in their work studied a relationship between bacterial counts collected by surface swabs and burn wound biopsy cultures.[87]They collected both the specimens either immediately prior to excision and grafting or during routine dressing changes. From their study they demonstrated that quantitative bacteriology by burn wound biopsy or surface swab does not aid in the prediction of sepsis or graft loss.

Loebl and colleagues demonstrated that the microorganisms obtained from the burn wound surface which was not excised showed poor correlation with that of tissue biopsy samples taken from the sub-eschar tissue. Freshwater and Su observed that the quantitative burn wound cultures should be clinically correlated with the burn wound infection and reported accordingly so that it serves as a useful guide in the management of burns patients with large TBSA burns.[79] Tahlan and colleagues also compared surface swabs and the burn wound biopsy cultures in their study in second and third degree burns.[83] They did not observe any difference in the isolates from the swabs and wound biopsies. Levine and colleagues compared swab and tissue biopsy specimens observed a numerical relationship between these two, whereby counts of

10^5 bacteria per gram of tissue in the biopsy sample and compared with counts of 10^6 bacteria obtained from surface swabs.[16]

McManus and colleagues found out that quantitative assay of tissue biopsy specimens was superior method to assess the microorganisms in the burn wound.[80] Sjoberg and colleagues in their study demonstrated that quantitative assay of tissue biopsies was a better method to assess sepsis to surface swabs but also noted that the procedure was very laborious regarding collection and analysis of the multiple samples.[86] Herruzo-Cabrera and colleagues observed that a method using semi-quantitative surface swab to distinguish between wound contamination and infection, using 10^5 organisms per gram as a threshold for the definition of infection by biopsy.[89] Bharadwaj and colleagues showed that blood cultures were also valuble in assessing burn wound sepsis when compared to that of swab or tissue biopsy cultures.[26] Their study showed that eventhough blood cultures were positive it proved to be a late sign in assessing invasive burn wound invasion.

For routine surveillance of the burn wound infections the apt sampling technique should be adopted according to the area of the burn wound as no single method provides a clinically reliable data on both the eschar and the areas excised. Superficial swab proves to be a most convenient and least invasive approach to provide the microbial flora

present in the wound surface. Moreover swabs can be taken from areas where skin is very thin to be biopsied like ears, eyes and phalanges. But a coordinated approach using quantitative tissue biopsy, blood, and urine samples can provide a best approach in assessing the burn patients with sepsis.[57] Deidre Church and colleagues also state that tissue biopsy samples should be sent for quantitative culture from burn wound in patients with sepsis.[57] They also state that tissue biopsy is necessary in order to diagnose unusual types of burn wound infections due to fungi and viruses.

BURN WOUND SAMPLING TECHNIQUES

There are various methods present for the surveillance of burn wound infections. The sample should be taken on regular basis, by a surface swab or by tissue biopsy.,[90,91,92,93] Multiple samples from several areas of the burn wound should be collected so that accurate determination of the amounts and the type of microorganisms isolated can be assessed irrespective of sampling technique. For the first few days to weeks after the injury samples should be collected frequently when the microbial flora is evolving. Then on, can be decreased to weekly, once the eschar has been excised, provided clinical signs of infection are not present.

SUPERFICIAL WOUND SAMPLES

Superficial wound samples consisting of qualitative and semiquantitative methods which are routinely employed in all clinical microbiological laboratories. Various techniques are employed including contact plates, collection of swabs and capillary guaze sampling.[16,86,93,94] Modern burn units universally rely on collection of swabs though other methods are described for only historical completeness. Surface swabbing must be done after the removal of dressings and surgical antibacterial agents and cleansing of the wound surface with 70% alcohol.[16,26,87,88] Surface swabs of the burn wound are a convenient and effective method for the routine collection of multiple samples.[16,88] The best method of surface swabbing is moving the sterile swab over an area of 1cm of the wound applying adequate pressure on the underlying tissues as to cause sufficient bleeding. Methods involving both dry and moist swabs have shown that moist swab technique provides better reproducibility.[84] Capillarity gauze sample collections are done by applying moistened guaze squares to the open wound surface for several minutes followed by inoculation into the agar plate.[93,94] This quantitative culture is more reproducible as the capillarity guaze provides more harvest of the resident bacteria.[93]

TISSUE BIOPSY

In an unexcised wound multiple samples from the subeschar tissue and quantitative culture has been historically the primary method for the accurate assessment of the unexcised burn wound.[82,85,95]After the study of Loebl and colleagues the quantitative biopsy culture was widely adopted into practice.[82,85] Initially the burn wound is cleaned with isopropyl alcohol, then two parallel incisions are made in the skin approximately 1 to 2 cm in length and 1.5 cm apart. With a help of a sterile forceps the tissue is elevated and with a scalpel the subcutaneous tissue is cut to a sufficient depth to obtain a small portion of the healthy underlying fat. Biopsy samples can also be collected by 3-mm punch biopsy.

Histopathological evaluation can detect bacteria, fungus by Periodic acid –Schiff (PAS) and Gomori methenamine silver (GMS), but cultures must be obtained to definitely identify and give sensitivity of the pathogen. Viruses like Herpes simplex virus (Giemsa) can be retrieved from the wound and identified by the presence of inclusion bodies in the microscope. Histology also guides in the staging of the burn wounds.[96,97]

The various stages used to diagnose burn wound infections are as follows:

STAGE I – COLONIZATION

a. Superficial – Microorganisms present only on burn wound surface
b. Penetrating – Variable depth of microbial penetration of eschar
c. Proliferating – Variable level of microbial proliferation at nonviable-viable tissue interface (subeschar space)

STAGE II – INVASION

a. Micro-invasion – Microorganisms present in viable tissue immediately subjacent to subeschar space

STAGE III - DEEP INVASION

a. Penetration of microorganisms to variable depth and expanse within viable subcutaneous tissues.
b. Micro-vascular involvement: Microorganisms within small blood vessels and lymphatics (thrombosis of vessels is common)

SAMPLING TECHNIQUES FOR OTHER PATHOGENS

Various methods are employed for the recovery of anaerobic bacteria, fungi and viruses from the burn wound. Anaerobic swab systems and pre-reduced anaerobic media that provide a suitable environment for the transport of inoculated surface swabs are commercially available.[98] To maintain the viability of anaerobic bacteria, the Copan VI-Pak agar gel collection system for a 24 hour transport.[99] However, tissue biopsy samples placed in saline moistened guaze maybe more reliable for the recovery of all anaerobic species from the burn wounds. For viruses and fungi, tissue biopsy for culture, immunofluorescence testing for viruses such as herpes simplex virus, and histology appear to be the most diagnostic methods.

MATERIALS AND METHODS

MATERIALS AND METHODS

Place of study	Thanjavur Medical College, Thanjavur
Study period	One year (June 2013 to 2014)
Collaborating Departments	Departments of Surgery and Plastic Surgery
Design of study	Prospective and Observational Study
Ethical Committee Clearance	Prior approval obtained from Ethical Committee
Informed Consent	Obtained from each patient
Sample	Tissue biopsy specimen

---------- ***** ----------

During the study period from June 2013 to July 2014, a total number of 246 tissue biopsy samples were collected from 83 patients with burns wound admitted at Thanjavur Medical college Hospital, Thanjavur. The samples were processed in the Central Service Laboratory, Microbiology Department, Thanjavur Medical College Hospital. For collection of the sample the following inclusion and exclusion criteria were considered.

INCLUSION CRITERIA

- All age groups and gender
- Patients with burn wounds ranging from 10% to 70% Total Body Surface Area Burns
- Patients with burn wound who are about to be grafted

EXCLUSION CRITERIA

- Patients with burns associated with chronic diseases like diabetes, etc
- Patients with burn wound less than 10% and more than 70%
- Patients with burn wound with clinical signs of septicaemia.

SPECIMEN COLLECTION

According to the above criteria the samples were collected. Samples from the burn wounds were collected by both surface swabbing and by tissue biopsy technique.

SURFACE SWABBING

To obtain a culture of burn surface, topical agents were first removed with a guaze soaked in sterile saline. The method of collection was deep swabbing, or aspiration of the bleb. Then the sample was collected by two sterile swab sticks. For dry wounds the swab was moistened with sterile saline. After the collection, the swab were immediately transported to the laboratory for further processing.

TISSUE BIOPSY SAMPLE

For quantitative cultures of biopsy samples, the wound was washed with saline soaked guaze pads to wash off the topical agents. The wound was biopsied by making two parallel incision by means of 11 blade, approximately 1 to 2 cm in length and 0.5 cm apart. Then the tissue was elevated by Alleys' Forceps and cut to a sufficient depth to obtain a small portion of the healthy underlying fat [82]. The tissue was placed directly into a sterilized pre-weighed homogenizer bag containing 1ml normal saline and the bag re-weighed. Weight of the tissue was obtained by subtracting the first weight from the second by the formula

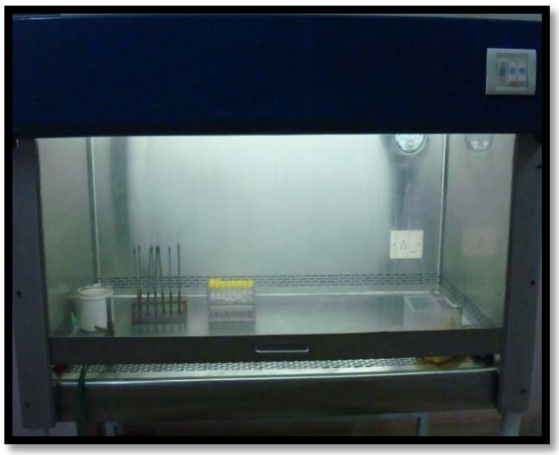
$$C = B - A$$

Where, C = weight of the tissue

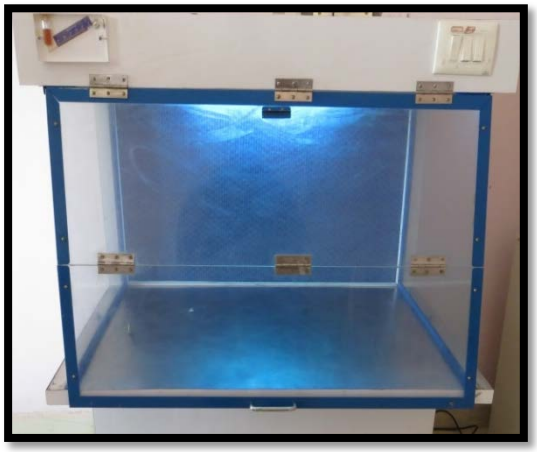
B = weight of bag with saline and

A = weight of bag with saline.

BIOSAFETY CABINET



LAMINAR FLOW HOOD



HOMOGENIZER BAG

DIGITAL BALANCE

MICROPIPETTES

This method of quantitative cultures were initially developed by Loebl[85].He collected the tissue sample by making two parallel incisions and by means of sterile tissue forceps elevated and biopsied the sample by means of a scalpel. Then he macerated the tissue and made several dilutions. Lawrence described as open method.[14] Baxter [10] homogenizes the tissue with the help of knife after suspending it in 1-2 ml of normal saline.Homogenization can also be done with a mortar and pastle.Robson et al[15] used a polytron homogenizer.Ganatra et al[100] used a punch biopsy forceps followed by homogenization in a Lab Blender Stomacher. Buchnan et al[101] used a sterile scalpel blade and forceps and cut into small pieces and homogenization carried out in a Ten Broeck tissue grinder.

SPECIMEN PROCESSING

The samples from both the surface swabbing and tissue biopsy collected from the burn patients were immediately transported to the laboratory for further processing.

SURFACE SWABBING

One of the swab sticks was used for direct gram staining. By the other swab stick, the sample was inoculated in MacConkey and blood agar plates and incubated for 18 to 24 hours at 37˚C. At the end of the incubation period, the plates were examined for the isolates.

TISSUE BIOPSY SAMPLE

The tissue placed in a homogenizer bag with 1ml of normal saline was homogenized in a tissue homogenizer (Lab Blender stomacher 80) for 30 seconds. Then with the homogenate, gram staining and quantitative bacteriology was performed simultaneously.

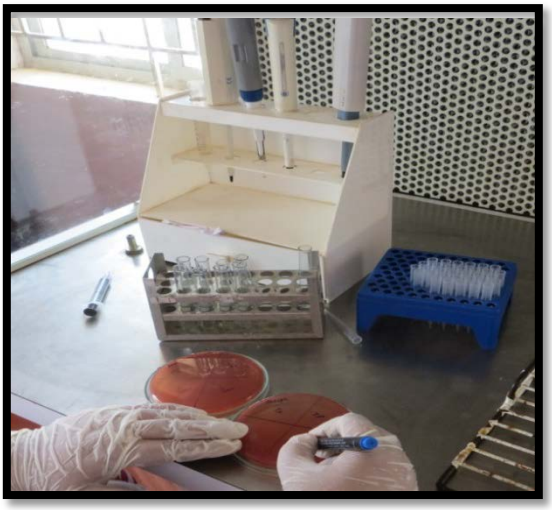
QUANTITATIVE BACTERIAL ASSAY

Then with the homogenate, several dilutions were made in the Laminar Flow Hood. Four test tubes containing 4.5 ml of normal saline each was taken. 0.5 ml of the homogenate is taken from the homogenizer bag with the help of a Micro pipette and added to the first test tube containing 4.5 ml of normal saline mixed well and the microtip was discarded. With a new microtip 0.5 ml was transferred to the second tube, mixed well and the micro tip was discarded. With a new microtip 0.5 ml is transferred to the third tube, mixed well and added to the fourth tube and then 0.5 ml taken from the fourth tube is discarded. Thus four dilutions were made, that is, 1/10, 1/100, 1/1000, 1/10000. Over three decades age, Loebl[82] and colleagues developed and evaluated a method for quantitative bacterial cultures of burn wound samples and have been widely adopted into practice. In this study a method is adapted similar to that described by Loebl with one difference is that Lab Bender Stomacher 80 has been used which ensures sterile and safe method.

LAB BLENDER STOMACHER 80

First direct Gram staining was done from the homogenate fluid. Pus cells, Gram positive and Gram negative organisms were noted. MacConkey agar plates and Blood agar plates were kept ready for inoculation. Each plate was divided into four quadrants and labelled as 1/10, 1/100, 1/1000, 1/10000. 0.01ml of homogenate is taken from each dilution tube and dropped in the MacConkey and Blood agar plates from a height of 2.5cm[100] ·Plates are then placed in the incubator with the lids slightly open for 15 minutes and then closed and incubated for 24 hours at 37˚C. After the incubation period, the surface forming colonies were noted for its morphological characteristics like opaque, moist or mucoid colonies, whether lactose fermenter or non-lactose fermenter, whether hemolytic or non-hemolytic and whether the isolate was single or multiple. Special characteristics were also looked for like swarming and for any specific odour. The number of colonies were counted by a hand lens and colony count per gram of tissue was calculated.

PROCESSING OF THE TISSUE BIOPSY SPECIMEN



The colony count per gram of tissue was obtained by the formula of Miles and Misra[102]

CFU/gm of tissue = C x D x V/W x 0.01

Where C = the total number of Colony forming units

D = is the dilution factor

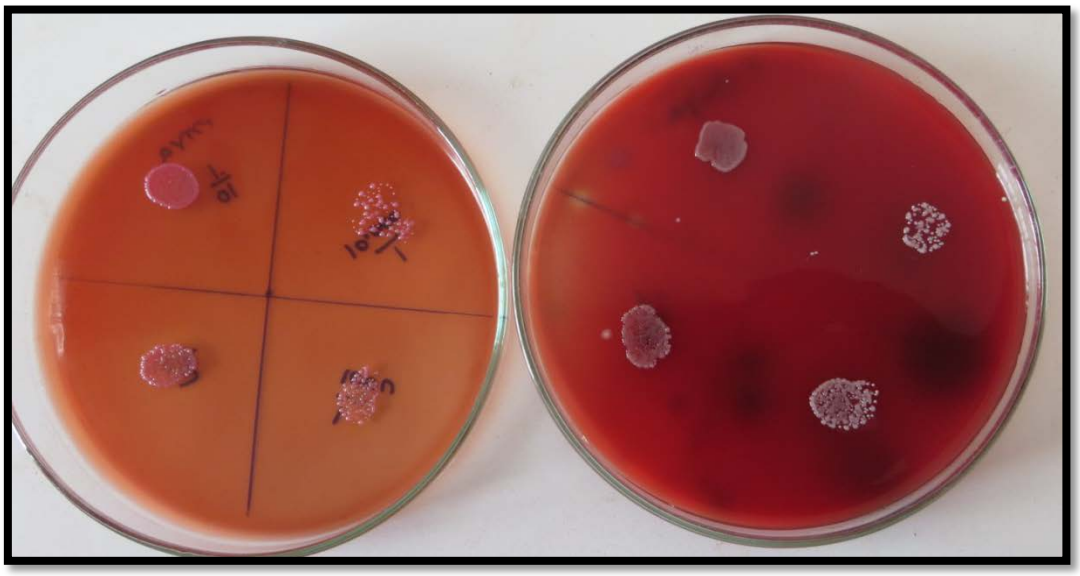
W = the weight of the tissue

V = the volume of normal saline

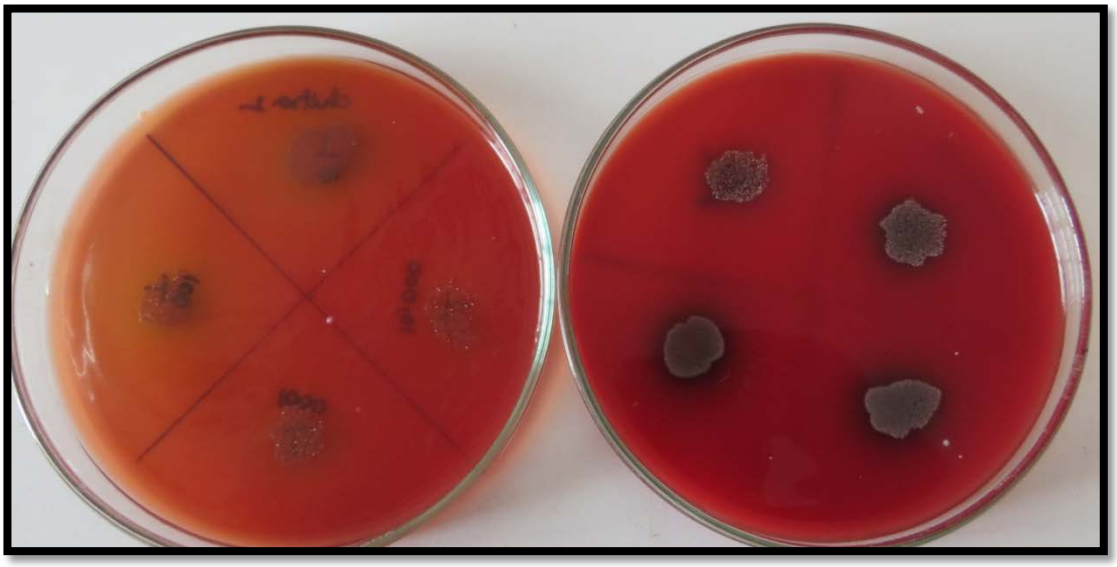
0.01 = the volume of the inoculum

Bacterial isolates were identified by adopting the procedures of Gram staining, motility and routine biochemical reactions. The Gram's staining was done from the colonies for both the swab specimen and the tissue homogenate fluid. The Gram positive and Gram negative organisms were noted and biochemical reactions performed accordingly. For the Gram positive organisms, Catalase test and coagulase test and other biochemical reactions was done. For Gram negative organisms Catalase test, oxidase test, motility test was done followed by Indole test, Methyl Red Test, Voges Proskauer Test, Citrate test, Urease test, nitrate reduction test, TSI test ,LAO decarboxylation, and OF test were done and results were recorded. On the basis of these tests, the organisms were

COLONIES OF KLEBSIELLA PNEUMONIAE



COLONIES OF PSEUDOMONAS AERUGINOSA



identified and noted. Then speciation was also done for each isolate. Quality control was also performed for all the standard tests.

IDENTIFICATION AND SPECIATION OF THE ORGANISM

1.Staphylococcus aureus

- Grampositive cocci in clusters
- Motility – non-motile
- Catalase – positive
- Slide Coagulase – positive
- Tube coagulase - positive
- B-Haemolysis - positive
- Indole production - negative
- Methyl red test – positive
- Voges Proskauer test – positive
- Citrate Utilization test – negative
- Urea hydrolysis test – positive
- TSI – A/A
- nitrate reduction test – positive
- OF test - fermentative
- Modified Oxidase test – negative

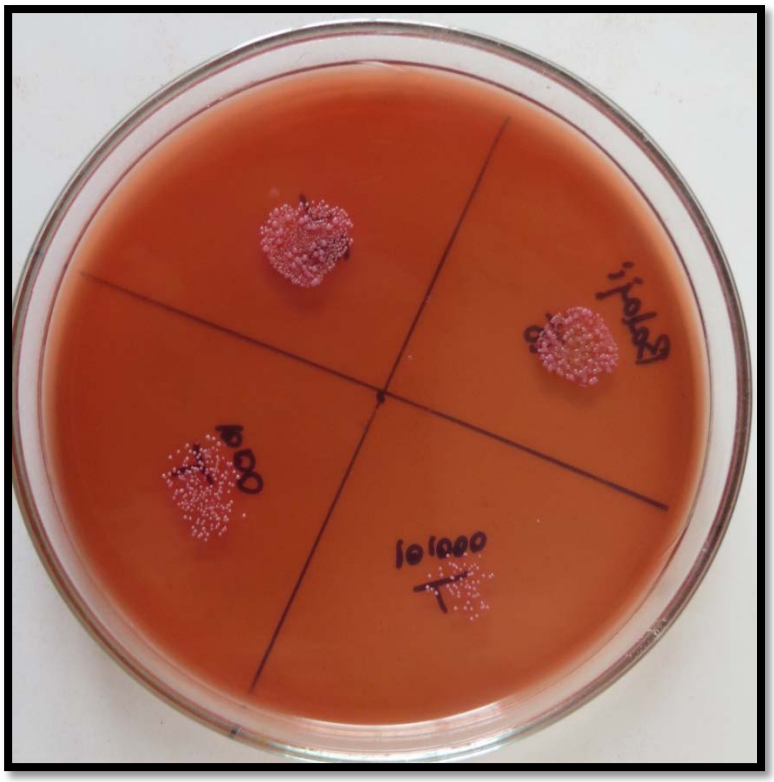
2. Klebsiella pneumonia:

- Gram negative rods
- Catalase test – positive
- Oxidase test – negative
- Motility test – non-motile
- Indole production - negative
- Methyl red test –negative
- Voges Proskauer test –positive
- Citrate Utilization test – positive
- Urea hydrolysis test – positive
- nitrate reduction test – positive
- Triple sugar iron agar test – A/A with gas
- LAO decarboxylation – lysine + , arginine - , Ornithine –

3. Klebsiella Oxytoca:

- Gram negative rods
- Catalase test - positive
- Oxidase test – negative
- Motility – non-motile
- Indole – positive
- Methyl red test – negative
- Voges Proskauer test –positive

MAC - STAPHYLOCOCCUS AUREUS COLONIES



MAC – KLEBSIELLA PNEUMONIAE COLONIES

- Citrate Utilization test – positive
- Urea hydrolysis test – positive
- Nitrate Reduction – positive
- Triple Sugar Iron agar test –A/A with gas
- LAO decarboxylation – lysine + , arginine - , Ornithine –

4. Escherichia coli

- Gram negative rods,
- Catalase test - positive
- Oxidase test - negative
- Motility – motile
- Indole – positive
- Methyl Red test– positive
- Voges Prauskauer test– negative
- Citrate utilization test–negative
- Urease test – negative
- Nitrate Reduction test - positive
- Triple Sugar Iron agar test –A/A
- LAO decarboxylation – lysine + , arginine - , Ornithine +

5. Proteus mirabilis:

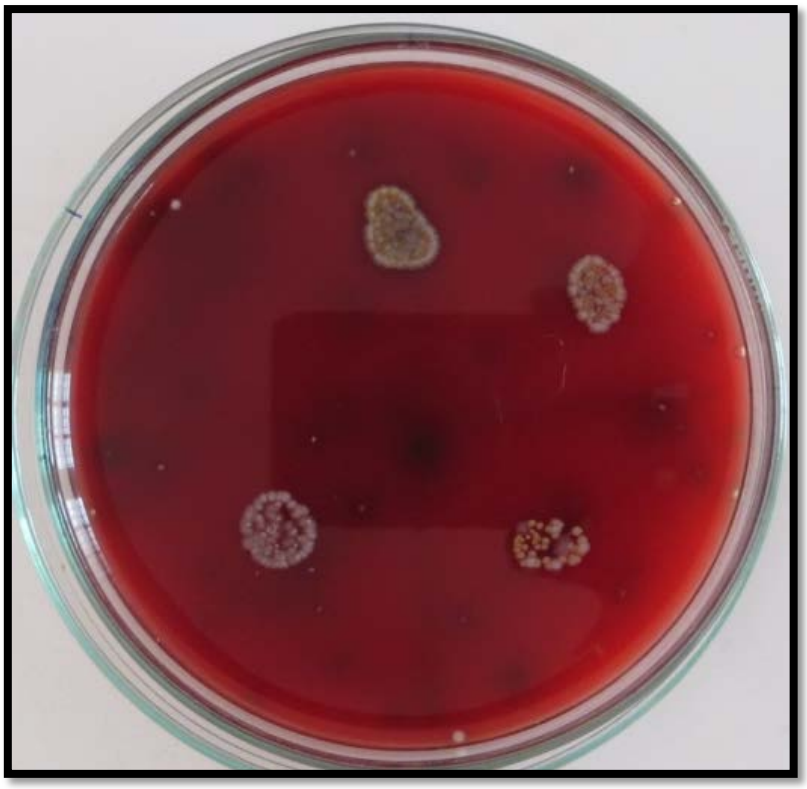
- Gram negative rods
- Catalase - positive

- Oxidase - negative
- Motility –actively motile
- swarming - positive
- Indole - negative
- Methyl red test – positive
- Voges Proskauer test – positive
- Citrate - negative
- Urease - positive
- Nitrate Reduction test – positive
- Triple sugar iron agar test – K/A with abundant H2S
- Phenyl alanine deaminase test – Positive
- LAO decarboxylation – lysine - , arginine - , Ornithine +

6. Enterobacter aerogenes

- Gram negative rods
- Catalase – positive
- Oxidase – negative
- Motility- motile
- Indole - negative
- Methy red test- negative
- Voges Proskauer – positive
- Citrate – positive

BLOOD AGAR – STAPHYLOCOCCUS AUREUS AND KLEBSIELLA PNEUMONIAE COLONIES



BLOOD AGAR – KLEBSIELLA PNEUMONIAE COLONIES

- Urease – negative
- Nitrate reduction test - positive
- Triple Sugar Iron agar test –A/A with gas
- LAO decarboxylation – lysine $_{+}$, arginine - , Ornithine +

7. Citrobacter freundii:

- Gram negative bacilli
- Catalase - positive
- Oxidase – negative
- Motility – motile
- Indole – negative
- Methyl red test – positive
- Voges Proskauer test – negative
- Citrate – positive
- Urease – positive
- Nitrate Reduction – positive
- Triple Sugar Iron agar test –A/A with H2S
- LAO decarboxylation – lysine $_{-}$, arginine + , Ornithine –

8. Pseudomonas aeruginosa:

- Gram negative bacilli
- Catalase – positive

- Oxidase – positive
- Motility – motile
- Indole – negative
- Methyl red test – negative
- Voges Proskauer test – negative
- Citrate utilization test – positive
- Urease test – negative
- Nitrate Reduction test – positive
- Triple Sugar Iron agar test –K/NC,
- LAO decarboxylation – lysine ˗ , arginine + , Ornithine –
- OF test - oxidative

9. Acinetobacter baumanni:

- Gram negative coccobacilli
- Catalase - positive
- Oxidase - negative
- Motility – non-motile
- Indole- negative
- Methyl Red test - negative
- Voges Proskauer test - negative
- Citrate utilization test - positive
- Urease test - negative

- Nitrate Reduction - negative
- Triple Sugar Iron agar test –K/NC,
- Growth at 42°C - positive
- OF test - oxidative
- LAO decarboxylation – lysine - , arginine + , Ornithine –
- 1% lactose - acid production
- 10% lactose - acid production

ANTI-MICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility test was done for all bacterial isolates as per CLSI guidelines by Kirby Disk Diffusion method on Muller Hinton agar.

STORAGE OF DRUGS

The antibiotic sensitivity was done by following the clinical and laboratory standards institute guidelines. The antimicrobial discs were stored at 4-8° C but the Beta lactum drugs like clavulanic acid and carbapenem group of drugs are stored in freezer ie, -20° since they are heat-labile agents.

The antimicrobial discs were brought to room temperature one to two hours before the procedure. The disc cartridge was always secured to

seal tightly after taking the disc out every time. All the disc cartridges were placed in air tight dry container.

PREPARATION OF TURBIDITY STANDARDS

Use of a standard inoculum size is important for culture purity and is accomplished by comparison of the turbidity of the organism suspension with a turbidity standard. McFarland turbidity standards, prepared by adding various volumes of 1% sulphuric acid and 1.175% barium chloride to obtain a barium sulphate solution with a specific optical density.

The most commonly used is the McFarland 0.5 Standard, which contains 99.5 ml of 1% sulphuric acid and 0.5 ml of 1.175 % barium chloride. The solution is dispensed into tubes comparable to those used for inoculum preparation which are sealed tightly and stored in the dark at room temperature.

The McFarland 0.5 Standard provides a turbidity comparable to that of a bacterial suspension containing approximately 1.5×10^8 CFU/ml. Matching turbidity using the unaided eye is facilitated by holding the bacterial suspension and McFarland tubes side by side and viewing them against a black-lined background.

PREPARATION OF INOCULUM

The pure inoculum was obtained by selecting the 4-5 representative colonies of same morphology inoculated in 4-5 ml of peptone water and allowing the organism to achieve good active growth (ie, mid logarithmic phase) by incubation at 37˚C for 2-6 hours. The growth was indicated by observable turbidity in the broth. The growth turbidity compared with 0.5 McFarland's Standard which corresponds to 150 million organisms/ml. If the bacterial suspension does not match the standard's turbidity, the suspension may be diluted or supplemented with more organisms as needed.

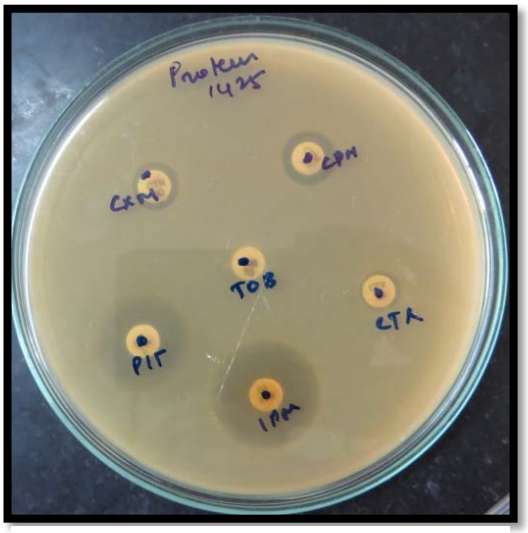
ANTI-BACTERIAL SUSCEPTIBILITY TEST PROCEDURE

15-20 ml of sterilized Mueller Hinton Agar medium was poured into each sterile petridishes and allowed to solidify. The test bacterial cultures were evenly spread over the media and excessive inoculum drained off. The panel of antimicrobial disc for Gram positive isolates like Ampicillin(10µg), Erythromycin(15µg), Cotrimoxazole(1.25/23.75µg), Doxycycline(10µg), Amikacin,(10µg), Gentamicin(10µg), Ciprofloxacin (10µg), Ofloxacin(30µg), Cephelexin(30µg), Ceftriaxone (30µg), Cefoxitin (30µg), Linezolid (30µg) and Vancomycin (30µg) were tested appropriately for each isolate. The panel of antimicrobial disc for Gram negative isoaltes like

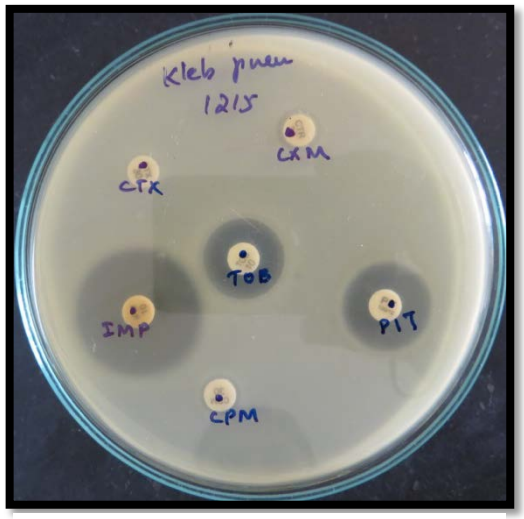
ANTIMICROBIAL SUSCEPTIBILITY TESTING

ESCHERICHIA COLI

MRSA



PROTEUS MIRABILIS



KLEBSIELLA PNEUMONIAE

Ampicillin (10µg), Tobramycin(10µg) , Cotrimoxazole (1.25/23.75µg), Amikacin (10µg), Gentamicin (10µg), Ciprofloxacin (10µg), Cephelexin (30µg), Cefotaxime (30µg), Ceftriaxone(30µg), Ceftazidime(30µg), Ceftazidime+clav(30µg),Piperacillin+Tazo(100/10µg), Aztreonam(30µg), and Imipenam(10µg) were tested. The discs were placed on agar plates and pressed down to ensure complete contact with the agar surface. Discs were distributed evenly so that they were not closer than 25 mm from centre to centre of the disc and incubated at 37° C for 16 – 18 hours. After incubation period, the zone of inhibition around each disc was measured and the results were interpreted.

READING AND INTERPRETATION OF RESULTS

After 16 -18 hours of incubation, each plate was examined for satisfactory streaking with uniformly circular zones of inhibition and semi confluent lawn of growth. The diameter of the zones of complete inhibition including the diameter of the discs was measured. The zones were measured using zone scale that was held on the back by inverting Petri plate. The Petri plate was held a few inches above a black, non - reflecting background and illuminated with reflected light. The zone margin showing no obvious visible growth that could be detected with unaided eyes was considered as the zone of inhibition . The sizes of the zones of inhibition were interpreted by referring to the CLSI standards

and reported as ‘Susceptible’, ‘Intermediate’ or ‘Resistant’ to the drugs that were tested.

CONTROL STRAINS USED WITH EACH BATCH ARE

- Escherichia coli ATCC 25922
- Pseudomonas aeruginosa ATCC 27853
- Staphylococcus aureus ATCC 25923

STATISTICAL ANALYSIS

All statistical analysis were performed using SPSS version with (p value) $p < 0.05$ accepted as statistically significant. Univariate analysis was used to compare the relationship between age, gender, burn depth, total body surface burn area and positive results of quantitative biopsy versus surface swab cultures. The Chi-square test was used for categorical variables, and Student’s t-test was used for continuous variables.

TABLE 3

ZONE SIZE INTERPRETATION CHART ACCORDING TO CLSI

PRIMARY USE

S.No	Antimicrobial agent	Symbol	Disc conc (µg)	Zone size in mm		
				Resistant	IMS	Sensitive
1	Ampicillin	AMP	10	<13	14-16	>17
2	Erythromycin	E	15	<13	14-22	>23
3	Cotrimoxazole	COT	1.25/ 23.75	<10	11-15	>16
4	Gentamicin	GEN	10	<12	13-14	>15
5	Amikacin	AK	30	<14	15-16	>17
6	Ciprofloxacin	CF	5	<15	16-20	>21
7	Cephalexin	CH	30	<14	15-17	>18
8	Ceftrioxone	CTR	30	<13	14-20	>21
9	Cefoxitin	CX	30	<14	15-17	>18

TABLE 4

ZONE OF INTERPRETATION CHART ACCORDING TO CLSI

PRIMARY BUT SELECTIVE DRUGS

S.No	Antimicrobial agent	Symbol	Discconc (µg)	Zone size in mm		
				R	IMS	S
1	Aztreonam	AT	30	<15	16-21	>22
2	Cefotaxime	CTX	30	<14	15-22	>23
3	Ceftazidime	CAZ	30	<14	15-17	>18
4	Doxycyclin	DO	30	<12	13-15	>16
5	Imipenem	IPM	10	<13	14-15	>16
6	Linezolid	LZ	30	-	-	>21
7	Ofloxacin	OF	5	<12	13-15	>16
8	Piperacillin/Tazobactum	PIT	100/10	<17	18-20	>21
9	Rifampicin	RIF	5	<16	17-19	>20
10	Tobramycin	TOB	10	<12	13-14	>15
11	Vancomycin	VA	30	-	-	>15
12	Ceftazidime/Clav	CAC	30/10	-	-	-

TABLE 5

ZONE OF INTERPRETATION CHART ACCORDING TO CLSI

SUPPLEMENTAL OR RESERVE DRUGS

S.No	Antimicrobial agent	Symbol	Discconc (µg)	Zone size in mm		
				R	IMS	S
1	Chloramphenicol	C	30 µg	12	13-17	18
2	Cefepime	CPM	30 µg	14	15-17	18
3	Ticaricillin	TI	75 µg	14	-	15
4	Netilmycin	NET	30 µg	12	13-14	15
5	Meropenam	MRP	10 µg	13	14-15	16
6	Teicoplanin	TEI	30 µg	10	11-13	14
7	Rifampicin	RIF	5 µg	16	17-19	20
8	Levofloxacin	LE	5 µg	15	16-18	19
9	Moxifloxacin	MO	5 µg	20	21-23	24
10	Quinupristin / Dalfopristin	RP	15 µg	15	16-18	19
11	Ticarcillin / Clavulanate	TCC	75/10 µg	14	15-19	20
12	Colistin	CL	10 µg	10	-	11
13	Polymyxin B	PB	300 units	11	-	12

DETECTION OF RESISTANT ISOLATES

Multidrug resistant organisms include Methicillin resistant staphylococcus aureus (MRSA), Exdended spectrum beta lactamase (ESBL) producing Gram negative bacteria, MDR pseudomonas and MDR Acinetobacter. Detection of multiple drug resistance organisms was done by various phenotypic methods approved by the CLSI[103]

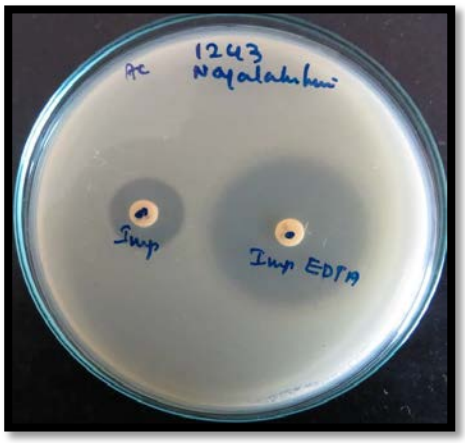
DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE

Detection of ESBL was done by Phenotypic confirmation test by double disk diffusion. This was done on Mueller Hinton agar with 0.5 McFarland standard of the organism. Lawn culture of the organism was made. A Ceftazidime (30 μg) disc and Ceftazidime/Clavulanic acid (30μg/10μg) are both kept well in contact with the agar surface. Plates were incubated at 37˚C for 24 hours. A 5 mm increase in zone of inhibition for Ceftazidime/Clavulanic acid was confirmed as ESBL.

DETECTION OF METALLO BETA LACTAMASES

Detection of MBL was done by Combined Disk Test using Imipenam (10μg) and Imipenam EDTA (10/750μg). The test inoculum was prepared with an overnight growth of each isolate, which was adjusted to a turbidity equivalent to 0.5 McFarland standard. The test organism was inoculated in MHA plate. The inoculum was allowed to

MULTIDRUG RESISTANT PATTERNS



MBL – ACINETOBACTER BAUMANNII

MBL – PSEUDOMONAS AERUGINOSA

MRSA – STAPHYLOCOCCUS AUREUS

MRSA – STAPHYLOCOCCUS AUREUS

ESBL – KLEBSIELLA PNEUMONIAE

ESBL – KLEBSIELLA PNEUMONIAE

dry for 5 minutes with lid in place. Then the Imipenam and Imipenam EDTA discs kept on the agar and incubated at 37 ° C for 24 hours.The increase in inhibition zone with IMP -EDTA disc by more than 7mm over that of IMP is MBL positive.

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Detection of MRSA is done by Cefoxitin disc diffusion test. Atleast three to five well isolated colonies of the same morphological type are selected from as agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a peptone water until it achieves the turbidity of the 0.5 McFarland standard containing approximately 1 x 10^8 CFU/ml. A lawn culture was done on the Mueller-Hinton agar plate and a Cefoxitin disc of concentration 30 μg was kept in the centre and pressed well to ensure good contact to the agar surface. Plates were incubated at 37 ° C for 18-24 hours and zone diameters measured. An inhibition zone diameter of $\leq$ 19mm was reported as oxacillin resistant and $\geq$ 20 mm was considered as oxacillin sensitive.

RESULTS

RESULTS AND OBSERVATIONS

During the study period from June 2013 to July 2014, a total number of 83 surface swabs and 209 tissue biopsy samples were collected from 83 burn patients admitted in the burns ward at Thanjavur Medical College Hospital, Thanjavur. Both the surface swabs and the tissue biopsy specimens were processed in the 24 hours Microbiology Diagnostic Laboratory, Thanjavur Medical College Hospital.

TABLE 6

AGE DISTRIBUTION

Age	No.of Cases (n=83)	%
<15	5	6.02
16 – 30	43	51.81
31 – 45	23	27.71
46 – 60	11	13.25
>60	1	1.20

Among the total population ,the age and sex distribution were studied in burn wound infections. Out of 83 cases, the most common age group affected was 16-30 years (51.81%), followed by the age group 31-45 years (27.71%). Least number of cases was seen in the age group of more than 60 years (1.20%).Table 6.

CHART -1

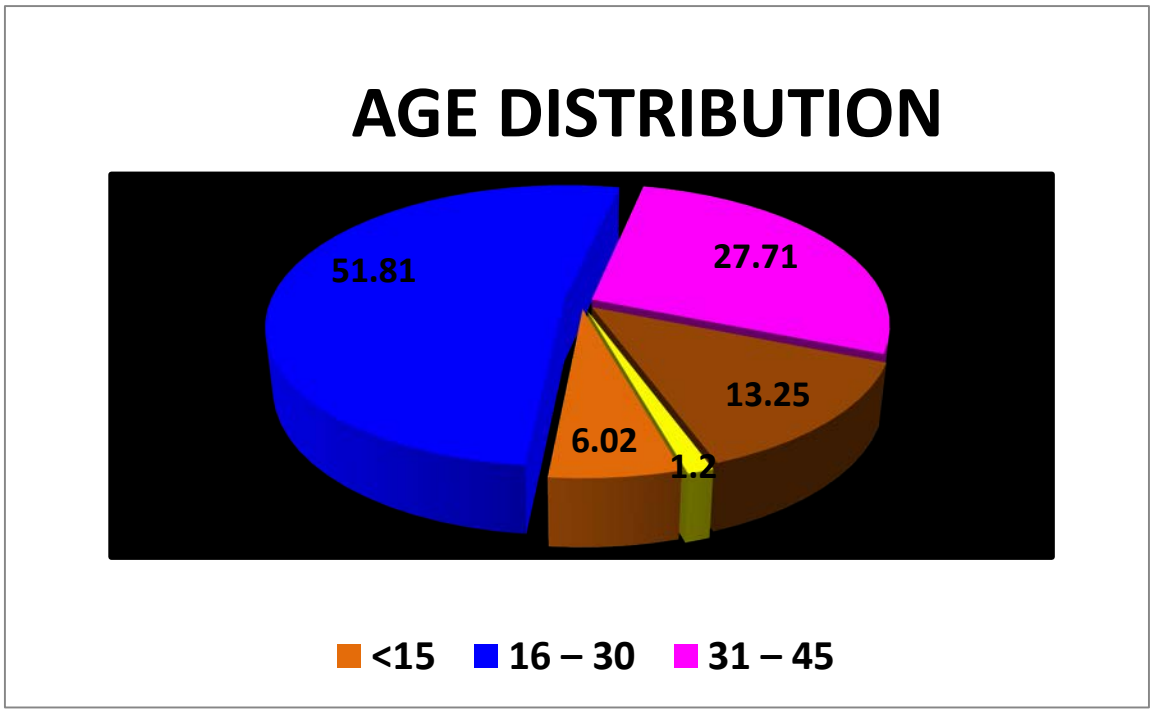


CHART -2

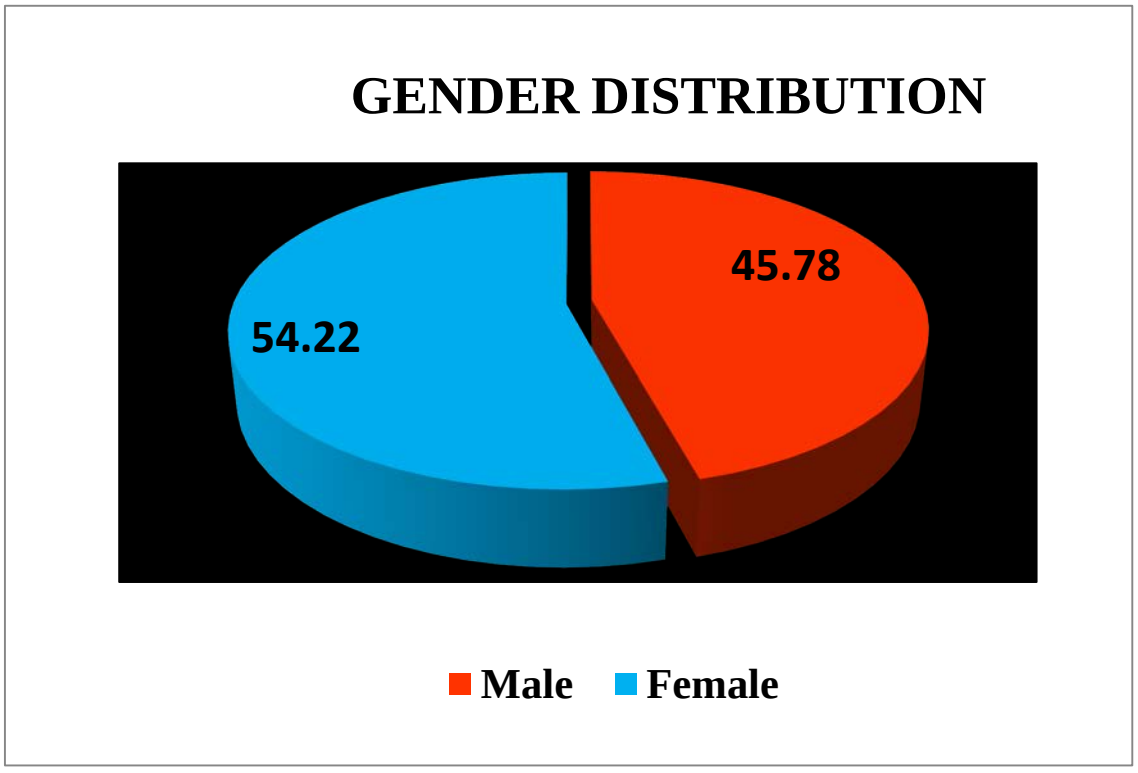


TABLE 7

GENDER DISTRIBUTION

Gender	No.of Cases (n=83)	%
Male	38	45.78
Female	45	54.22

Out of 83 cases, 45 cases were females (54.22%) and 38 cases were males (45.78%). Table 7

TABLE 8

PREVALENCE OF TOTAL BODY SURFACE AREA BURNS

TBSA burns	No. of cases (n=83)	Percentage
10-20%	26	31.33
21-30%	18	21.69
31-40%	11	13.25
41-50%	05	6.02
51-60%	17	20.48
61-70%	06	7.23

The burn wound infections were studied in relation to extent of burns and degree of burns. The extent of burns is expressed as total body surface area burns (TBSA) and marked as percentage of burns. Out of 83 cases, 26 persons were between 10-20% TBSA burns (31.33%) followed by 18 cases between 21-30% TBSA burns (21.69%). 11 cases (13.25%) were between 31-40% TBSA burns,6 cases (7.23%) were between 61-70% TBSA burns, and 17 cases (20.48%) were between 51-60%. Least number of cases were between 41-50% TBSA burns ie 5 cases (6.02%) Table 8

CHART - 3

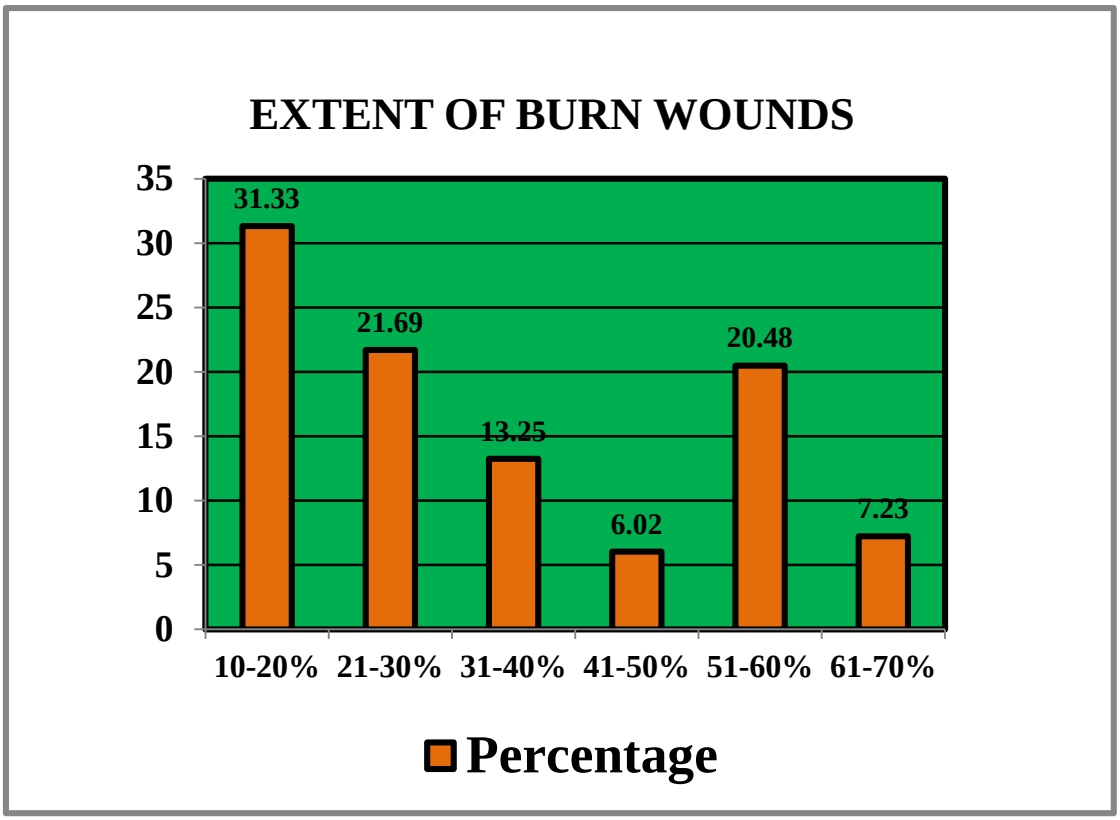


CHART - 4

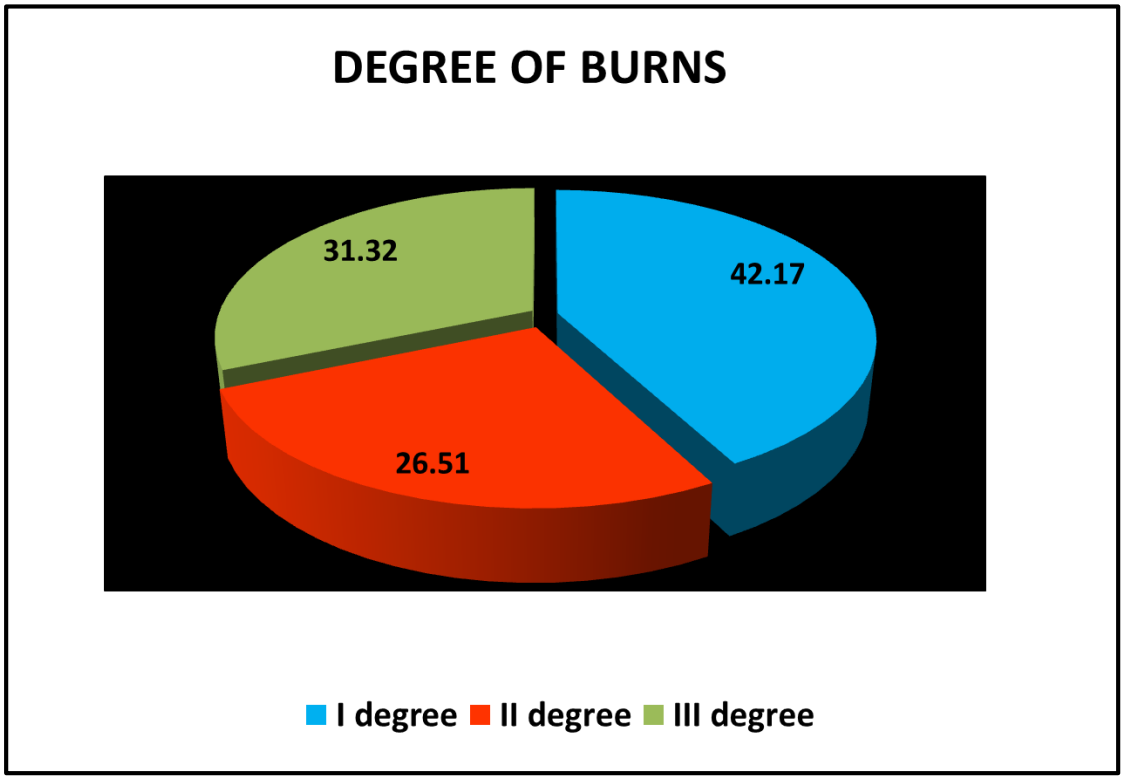


TABLE 9

DISTRIBUTION OF DEGREE OF BURNS.

Degree	No.of Cases (n=83)	%
I	35	42.17
II	22	26.51
III	26	31.32

The burn wounds were studied according to the degree of burns attained depending upon the depth of skin involvement. Out of 83 cases, 35 cases had I degree burns (42.17%) followed by 26 cases who had III degree burns (31.32%) and 22 cases (26.51%) had II degree burns. Table 9

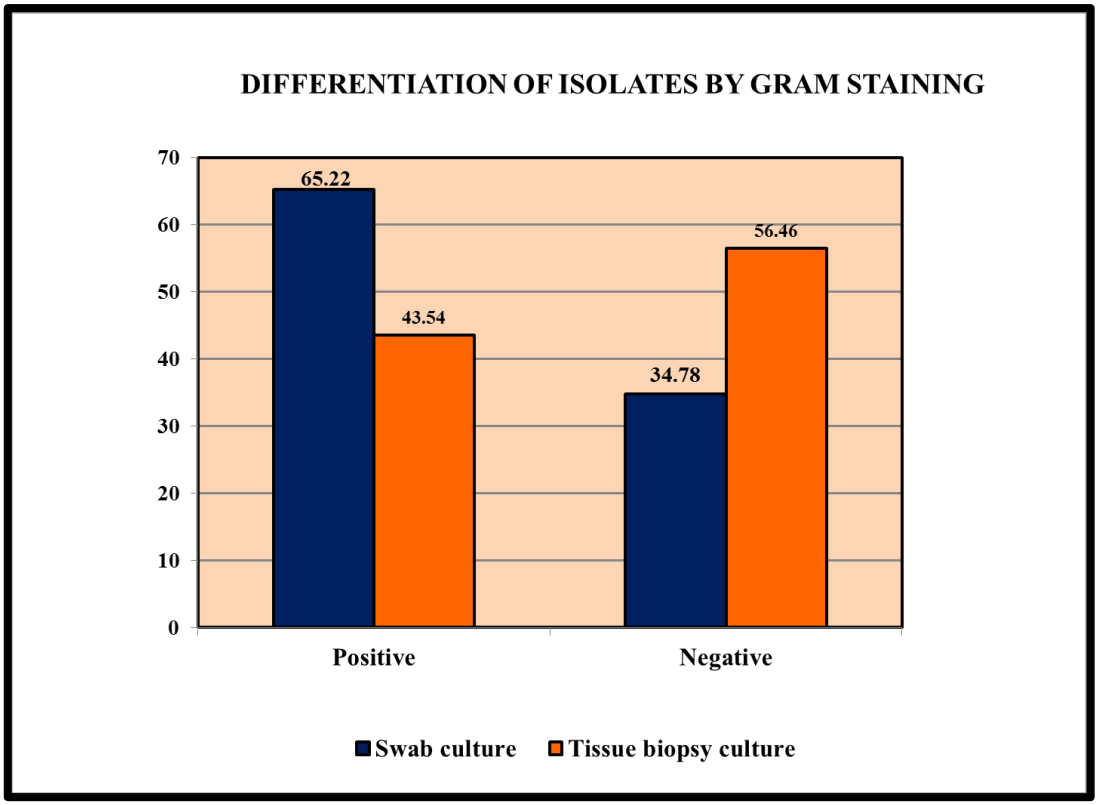
TABLE 10

DISTRIBUTION OF ISOLATES BY GRAM STAINING

Gram staining	Swab culture (n=69)	%	Tissue biopsy culture (n=294)	%
Positive	45	65.22	128	43.54
Negative	24	34.78	166	56.46

The isolates were differentiated according to gram staining procedure into gram positive and gram negative organisms. The distribution of isolates by gram staining were compared between the swab culture isolates and the tissue culture isolates. Out 69 swab culture isolates, 45 samples (65.22%) were gram positive and 24 samples (34.78%) were gram negative. Out of 294 isolates obtained from the tissue biopsy culture, 128 samples were gram positive (43.54%) and 166 samples were gram negative (56.46%). The surface swabbing showed gram positive predominance of organisms while in the tissue biopsy culture there was gram negative predominance. Table 10

CHART - 5



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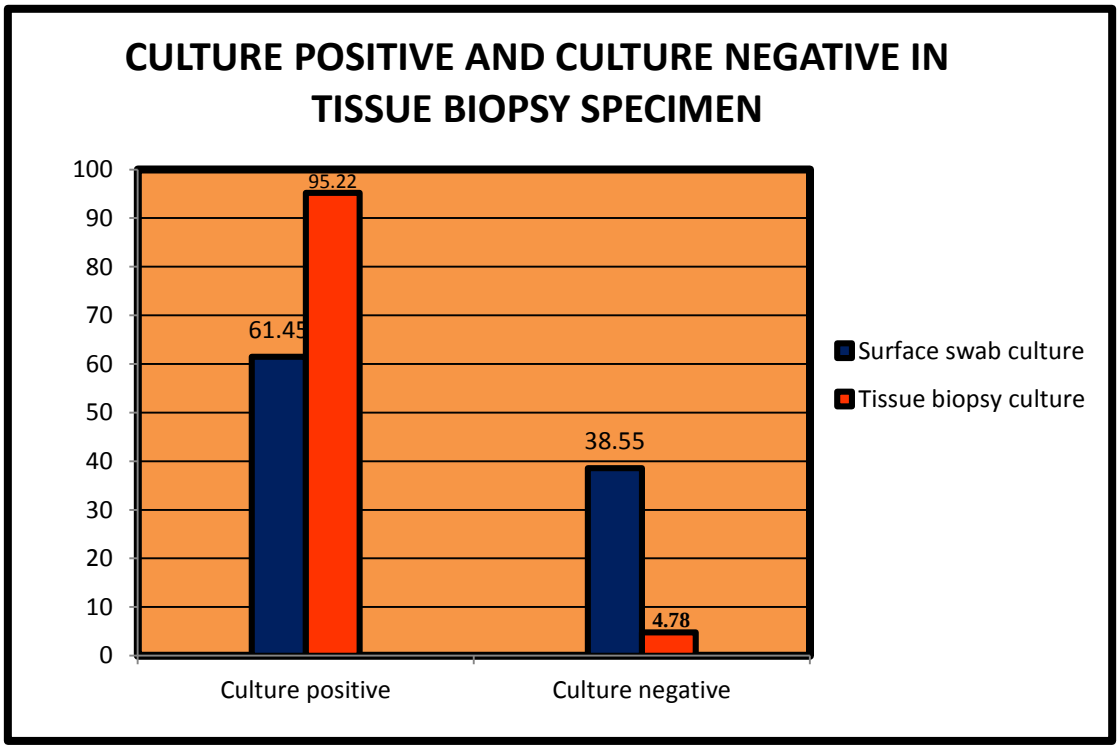


TABLE 11

CORRELATION BETWEEN SURFACE SWABBING AND TISSUE BIOPSY CULTURE

Specimen	Surface swab culture (n=83)	%	Tissue Biopsy culture (n=209)	%
Culture positive	51	61.45	199	95.22
Culture negative	32	38.55	10	4.78

Out of 83 surface swab cultures, 51 cases showed culture positive (61.45%) and 32 cases were culture negative (38.55%). Out of 209 tissue biopsy samples, 199 cases showed culture positive (95.22%) and 10 cases were culture negative (4.78%). (Table 11)

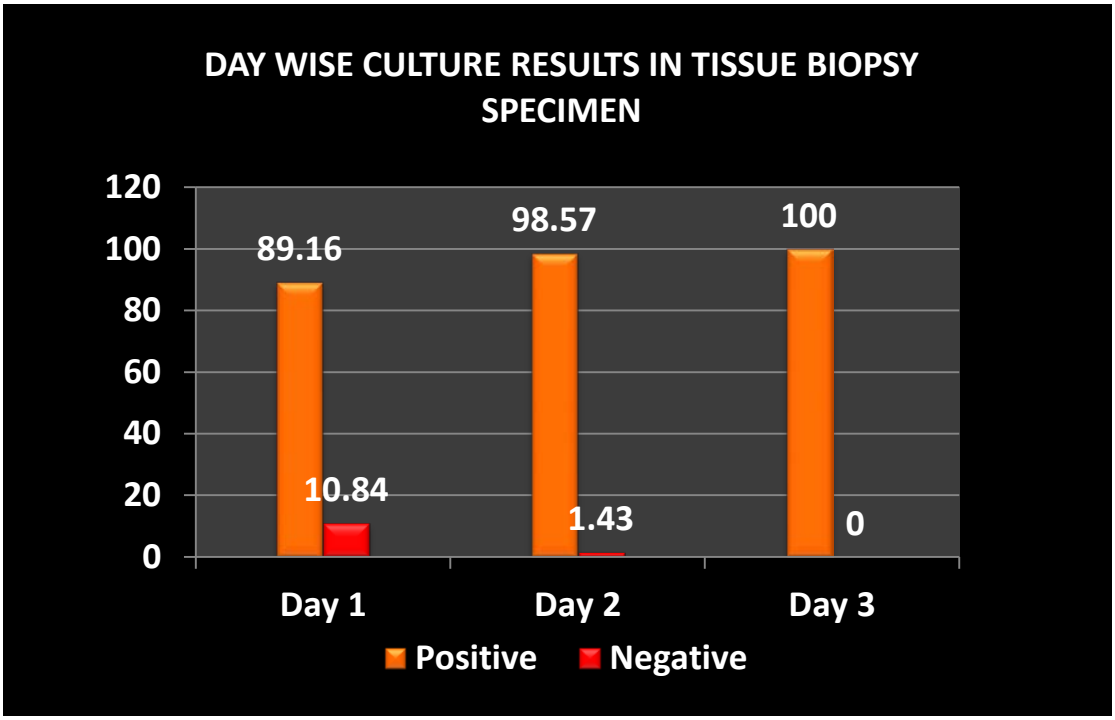
TABLE 12

CULTURE POSITIVE AND NEGATIVE CASES IN TISSUE BIOPSY SPECIMENS

Tissue Biopsy (n=209)	Day 1	%	Day 4	%	Day 9	%
Positive	74	89.16	69	98.57	56	100
Negative	09	10.84	01	1.43	0	0
Total	83		70		56	

In the tissue biopsy specimens, out of 83 samples taken on Day 1, 74 sampleswere culturepositive (89.16%), out of 70 samples taken on Day 4, 69 samples were culture positive (98.57%) and out of 56 samples taken on Day 9, 56 samples were culture positive (100%). Table 12

CHART - 7



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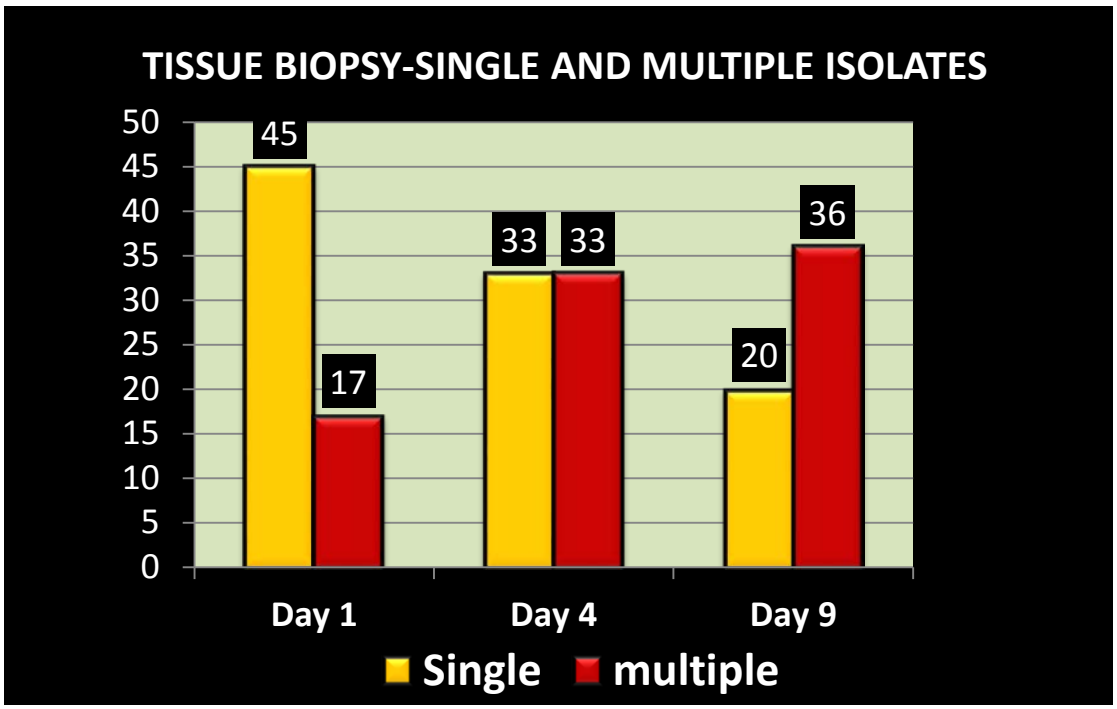


TABLE 13

PREVALENCE OF SINGLE AND MULTIPLE ISOLATES IN TISSUE BIOPSY

No. of isolates	Day 1	Day 4	Day 9	Total
Single	45	33	20	98
multiple	17	33	36	86

Among the 294 isolates from the tissue biopsy specimens, 45 single isolates and 17 multiple isolates were found on samples taken on Day 1, 33 single isolates and 33 multiple isolates from sample taken on Day 4, and 20 single isolates and 36 multiple isolates from sample taken on Day 9.(Table 13)

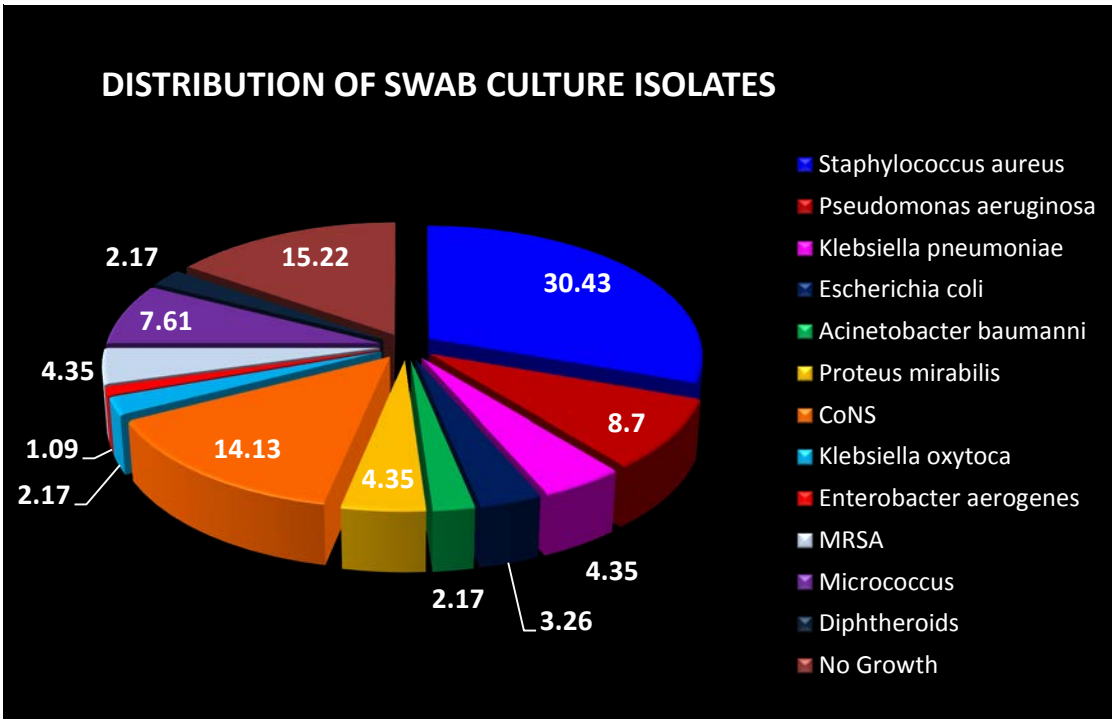
TABLE 14

PREVALENCE OF ORGANISMS IN SWAB CULTURE TECHNIQUE

Isolates	No.of isolates (n=92)	percentage
Staphylococcus aureus	28	30.43
Pseudomonas aeruginosa	8	8.70
Klebsiella pneumoniae	4	4.35
Escherichia coli	3	3.26
Acinetobacter baumanni	2	2.17
Proteus mirabilis	4	4.35
CoNS	13	14.13
Klebsiella oxytoca	2	2.17
Enterobacter aerogenes	1	1.09
MRSA	4	4.35
Micrococcus	7	7.61
Diphtheroids	2	2.17
No Growth	14	15.22

In this study, surface swabbing showed Staphylococcus aureus (30.43%) was the commonest organism from the burn wounds followed by CoNS (14.13%), Pseudomonas aeruginosa (8.70%), Klebsiella pneumonia (4.35%), Proteus mirabilis (4.35%) and MRSA (4.35%), Escherichia coli (3.26%), Acinetobacter baumanni (2.17%), Klebsiella oxytoca (2.17%), Enterobacter aerogenes (1.09%). Micrococcus was isolated in 7 cases (7.61%). Diphtheroids was found in (2.17%). No growth was reported in 15.22%. (Table 14)

CHART - 9



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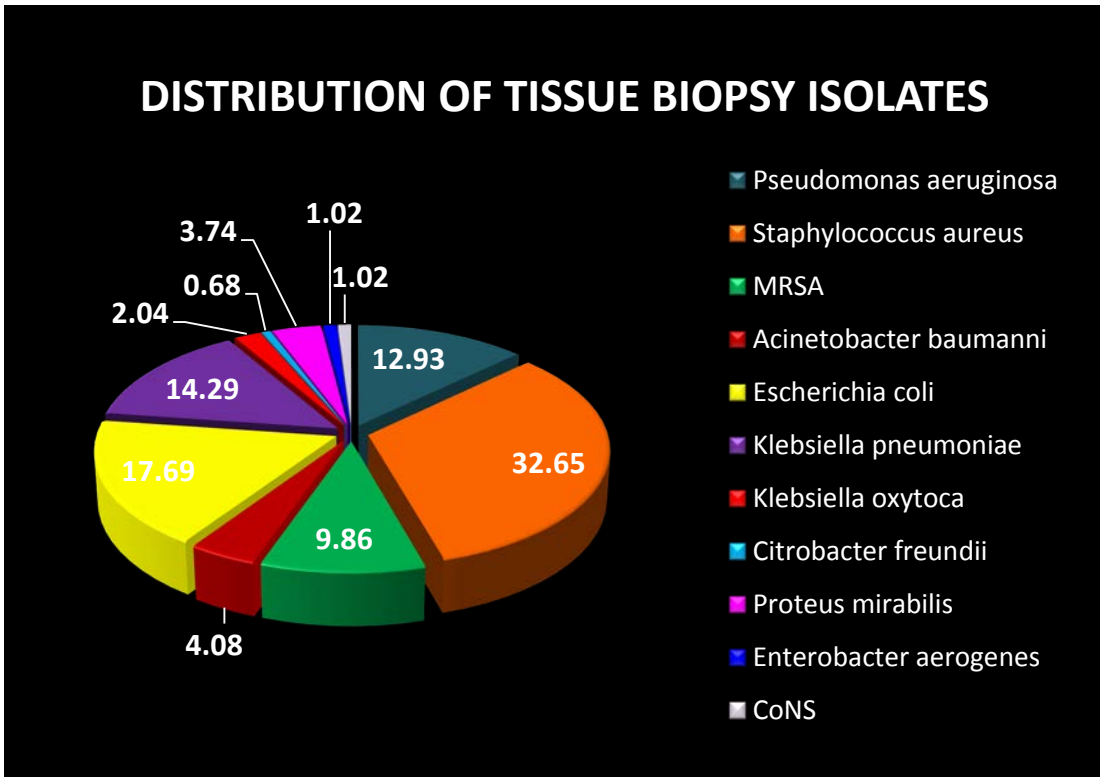

TABLE 15

PREVALENCE OF ORGANISMS IN TISSUE BIOPSY CULTURE

Isolates	No. of isolates (n=294)			Total	%
	Day 1	Day 4	Day 9		
Pseudomonas aeruginosa	14	13	11	38	12.93
Staphylococcus aureus	35	32	29	96	32.65
MRSA	10	10	9	29	9.86
Acinetobacter baumanni	5	5	2	12	4.08
Escherichia coli	9	20	23	52	17.69
Klebsiella pneumoniae	15	13	14	42	14.29
Klebsiella oxytoca	2	3	1	6	2.04
Citrobacter freundii	1	1	0	2	0.68
Proteus mirabilis	4	4	3	11	3.74
Enterobacter aerogenes	1	1	1	3	1.02
CoNS	1	1	1	3	1.02
No Growth	9	1	0	10	0

The predominant organism isolated in the tissue biopsy culture was Staphylococcus aureus 32.65% followed by Escherichia coli 17.69% and Klebsiella pneumonia 14.29%. Pseudomonas aeruginosa was 12.93%, MRSA was 9.86%, Acinetobacter baumanni was 4.08%, Klebsiella oxytoca 2.04%,Proteus mirabilis 3.74%, Citrobacter freundii 0.68%,Enterobacter aerogenes 1.02%, CoNS was 1.02%. No growth was reported in 10 cases and was predominantly noted in the Day 1 sample (9 cases) and I case was reported in Day 4 sample. (Table-15)

TABLE 16

COMPARISON OF ISOLATES BY SWAB AND TISSUE BIOPSY CULTURES

organisms	Swab culture (n=92)	%	Tissue biopsy (n=294)	%
Staphylococcus aureus	28	30.43	96	32.65
Pseudomonas aeruginosa	8	8.70	38	12.93
Klebsiella pneumoniae	4	4.35	42	14.29
Escherichia coli	3	3.26	52	17.69
Acinetobacter baumanni	2	2.17	12	4.08
Proteus mirabilis	4	4.35	11	3.74
CoNS	13	14.13	3	1.02
Enterobacter aerogenes	1	1.09	3	1.02
Klebsiella oxytoca	2	2.17	6	2.04
MRSA	4	4.35	29	9.86
Citrobacter freundii	0	0	2	0.68
Micrococcus	7	7.61	0	0
Diphtheroids	2	2.17	0	0
No Growth	14	15.22	10	0

While comparing both the swab and tissue cultures, both showed predominance of Staphylococcus aureus. It should be noted that **CoNS, Micrococci, Diphtheroids and culture negatives were reported only in surface swabbing but not in tissue biopsy cultures**. CoNS was isolated only in three cases. There were no Micrococci and Diphtheroids reported in the tissue biopsy cultures.Moreover, Klebsiella pneumonia and Escherchia coli were more common colonizers in the tissue biopsy cultures. On comparison of 92 surface swab cultures and 294 tissue biopsy cultures,there was moderate concordance for Staphylococcus aureus, low concordance for of Klebsiella oxytoca, Enterobacter aerogenes, and Proteus mirabilis.(Table 16)

CHART – 11

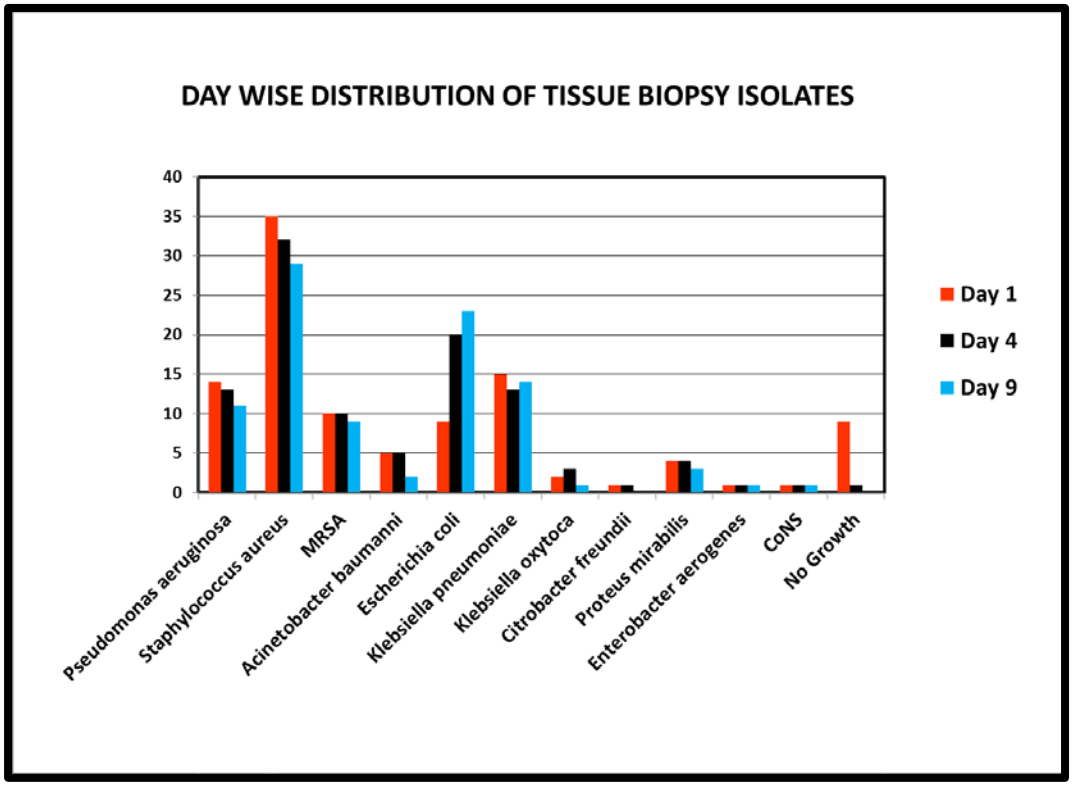

CHART – 12

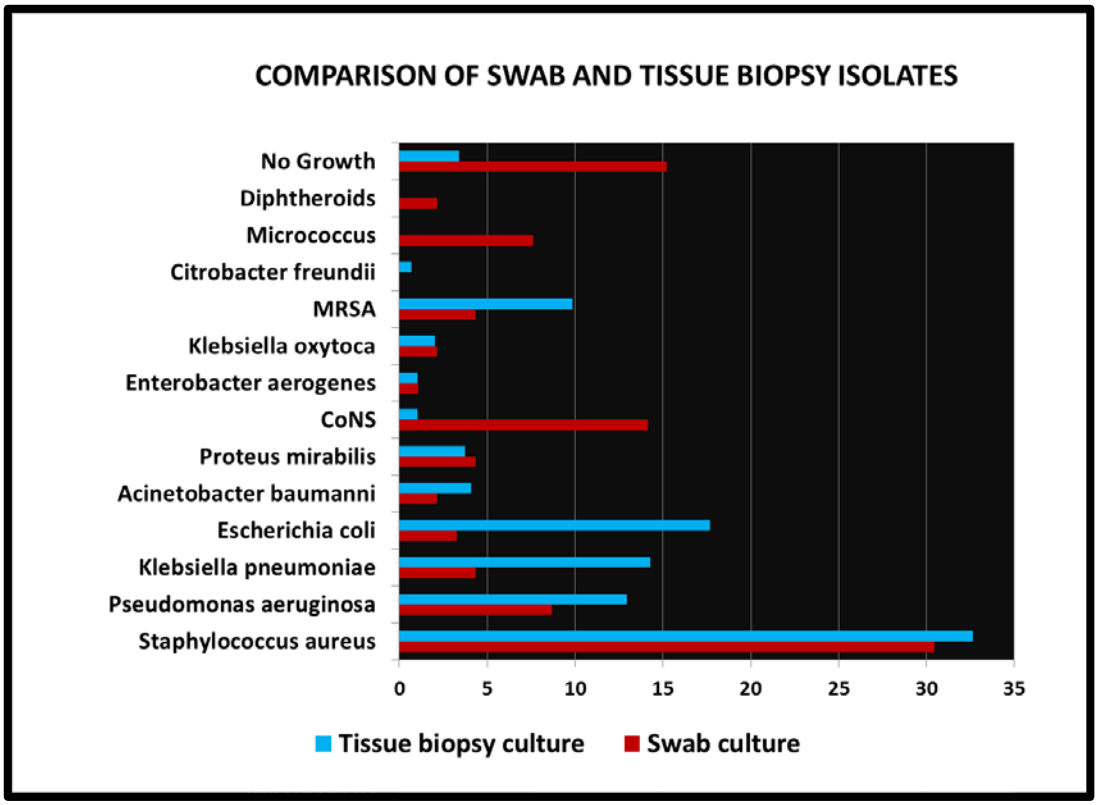

TABLE 17

DISTRIBUTION OF MEAN BACTERIAL COUNTS OF THE ISOLATES

Organisms	No. of isolates	Bacterial counts Mean ($\sim$ x 10^5)
Staphylococcus aureus	96	4.40
Pseudomonas aeruginosa	38	3.57
Klebsiella pneumonia	42	1.11
Escherichia coli	52	0.89
MRSA	29	4.78
Acinetobacter baumanni	12	1.75
Proteus mirabilis	11	2.54
Klebsiella oxytoca	6	0.54
Citrobacter freundii	2	2.35
Enterobacter aerogenes	3	2.40

Out of the 294 isolates, the bacterial counts were calculated for each organism isolated. Among the isolates, Staphylococcus aureus (96) showed a mean bacterial count of 4.40 x 10^5, Pseudomonas aeruginosa (38) was 3.57 x 10^5, Klebsiella pneumonia (42) was 1.11 x 10^5, MRSA (29) was 4.78 x 10^5, Acinetobacter baumannii (12) was 1.74 x 10^5, Proteus mirabilis (11) was 2.54 x 10^5, Citrobacter freundii (2) was 2.35 x 10^5, and Enterobacter aerogenes (3) was 2.40 x 10^5. Eshcherichia coli (52) and Klebsiella oxytoca (6) showed mean bacterial counts of 0.89 and 0.54 respectively which was less than 10^5 CFU/gm of tissue. Table 17.

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CHART - 13

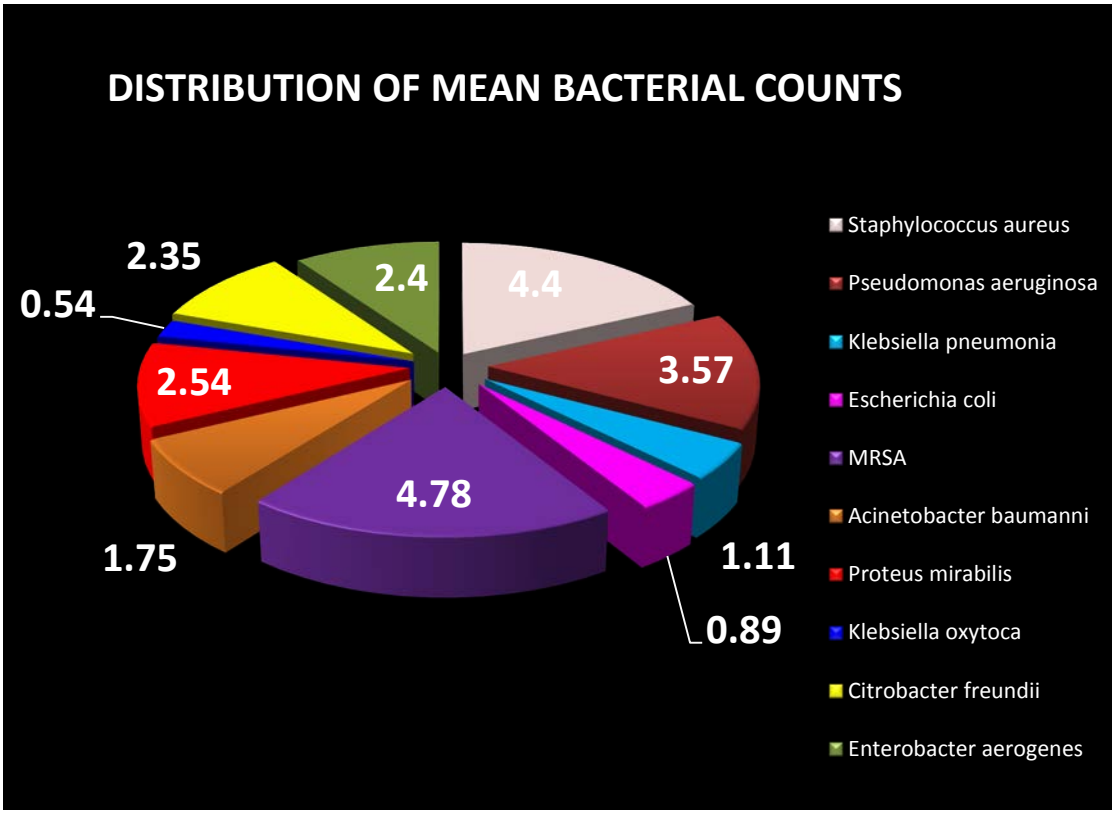


CHART - 14

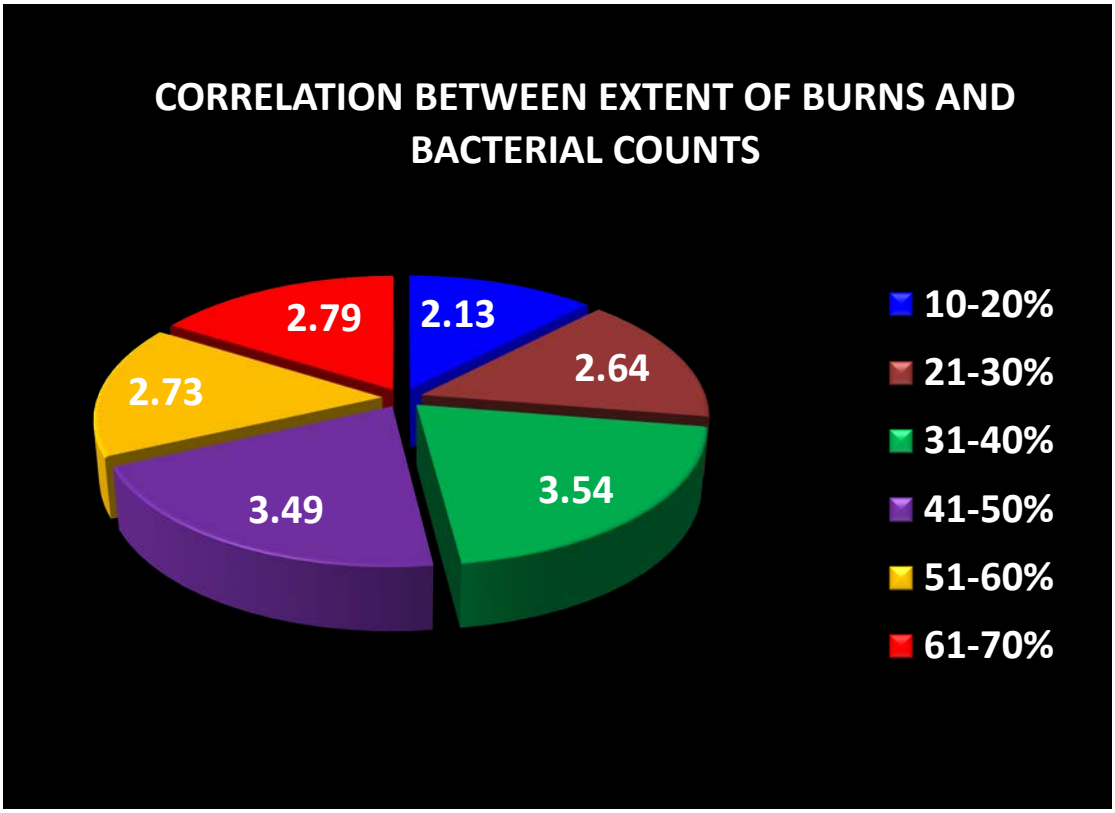


TABLE 18

CORRELATION BETWEEN BURN WOUND EXTENT AND BACTERIAL COUNTS

TBSA burns	No. of cases (n=83)	Bacterial counts (mean)
10-20%	26	2.13
21-30%	17	2.64
31-40%	11	3.54
41-50%	6	3.49
51-60%	17	2.73
61-70%	6	2.79

The mean bacterial counts were calculated in relation to the extent of burns and also to the degree of burns. The mean bacterial counts were higher in 31-40% TBSA burns (3.54) followed by 41-50% TBSA burns (3.49). The bacterial counts for 10-20% was 2.13, 21-30% was 2.64, 51-60% was 2.73% and 61-70% was 2.79%. There was 26 cases with 10-20% TBSA burns,17 cases with 21-30% and 51-60% TBSA burns, 11 cases with 31-40% TBSA burns and 6 cases with 41-50% and 61-70% TBSA burns. (Table 18)

TABLE 19

CORRELATION BETWEEN DEGREE OF BURNS AND BACTERIAL COUNTS

Degree of burns	No. of cases (n=83)	Bacterial counts (Mean)
I degree	35	2.21
II degee	22	3.68
III degree	26	2.88

According to the degree of burns, 35 cases were with I degree burns, 22 cases with II degree burns and 26 cases with III degree burns. The mean bacterial counts was higher in II degree burns which was 3.68, followed by III degree which was 2.88 and I degree with 2.21. (Table 19)

CHART - 15

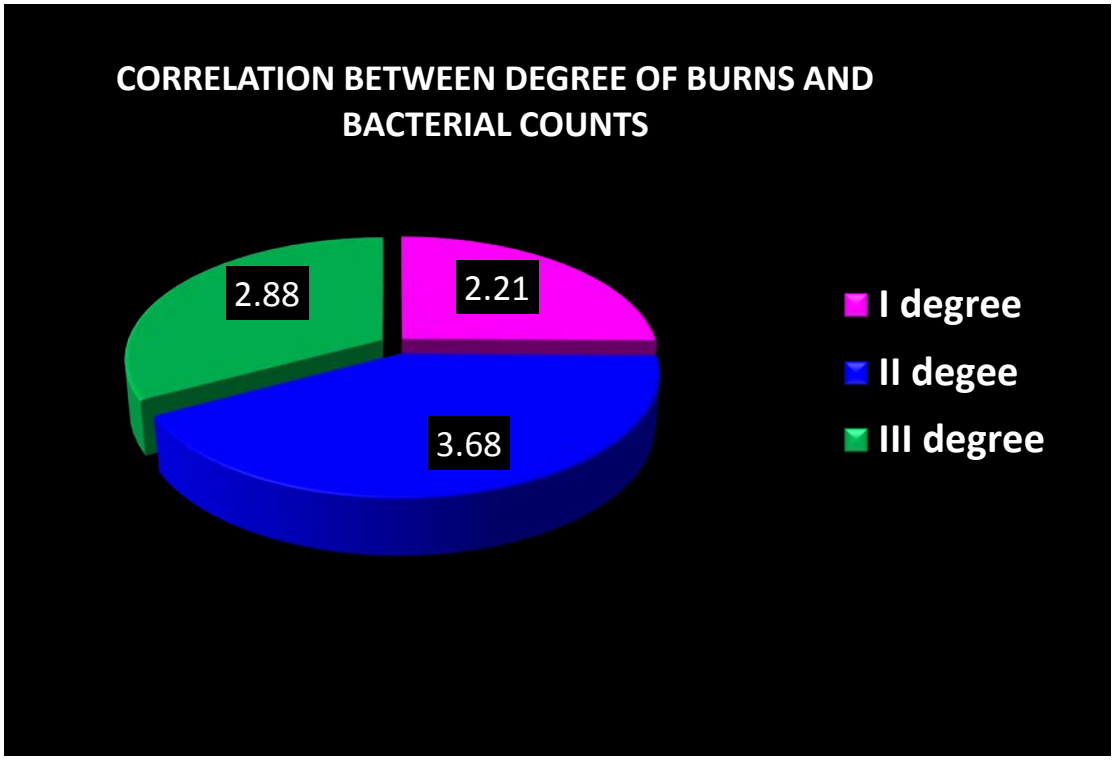


CHART - 16

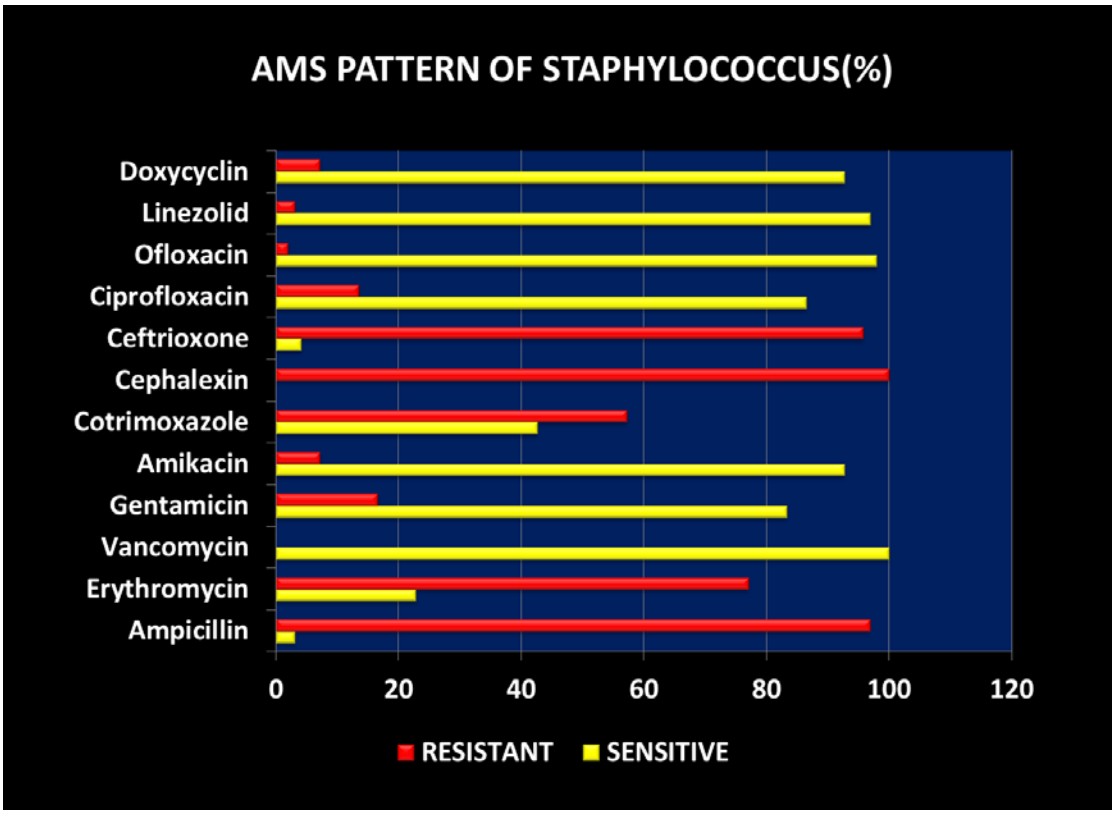


TABLE 20

CHARACTERISTICS OF SURFACE AND BIOPSY CULTURE POSITIVE BURN PATIENTS

Characteristics		Cases	Mean ± SD		P value
			Surface culture positive cases	Biopsy culture positive cases	
Age	<15	5	3	5	0.001
	16-30	43	22	42	
	31-45	23	15	22	
	46-60	11	8	9	
	>60	1	1	1	
Gender	Male	38	20	28	0.02
	Female	45	20	33	
Burn depth	I degree	35	18	18	0.31
	II degree	22	20	22	
	III degree	26	25	26	
TBSA burns	10 – 20%	26	12	25	0.008
	21 – 30%	17	12	15	
	31 – 40%	11	9	11	
	41 – 50%	6	4	6	
	51 – 60%	17	9	17	
	61 – 70%	6	4	6	

In the characteristic analysis, burn depth showed no significant association with the results of quantitative biopsy cultures. As for gender, there was significant association between the positive biopsy results and the female gender ($p<0.05$). Age group showed a significant association with the results of biopsy cultures with the age group between 16 – 30 ($p<0.05$). There was significantly lower level of TBSA in patients with positive surface swab cultures compared with that biopsy cultures ($p<0.05$). (Table 20)

TABLE 21

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCUS AUREUS

Antimicrobials	Staphylococcus aureus (n=96)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	3	3.13	93	96.87
Erythromycin	22	22.92	74	77.08
Vancomycin	96	100	0	0
Gentamicin	80	83.33	16	16.67
Amikacin	89	92.71	7	7.29
Cotrimoxazole	41	42.71	55	57.29
Cephalexin	0	0	96	100
Ceftrioxone	47	4.17	49	95.83
Ciprofloxacin	83	86.46	13	13.54
Ofloxacin	94	97.92	2	2.08
Linezolid	93	96.87	3	3.12
Doxycyclin	89	92.71	7	7.29

Antimicrobial susceptibility testing was carried out for all bacterial isolates as per CLSI guidelines by Kirby Bauer disk diffusion method. The antibiogram of Staphylococcus aureus showed sensitivity to Ampicillin (3.13%), Erythromycin (22.92%), Vancomycin (100%), Gentamicin (83.33%), Amikacin (92.71%), Cotrimoxazole (42.71%), Ceftrioxone (4.17%), Ciprofloxacin (86.46%), Ofloxacin (97.92%), Linezolid (96.87%) and Doxycyclin (92.71%). Total resistant patterns were noted in Cephelexin (100%) and relative resistant patterns were noted in Ampicillin (96.87%). Table 21

TABLE 22

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF MRSA

Antimicrobials	MRSA (n=29)			
	Sensitive	Percentage	Resistant	percentage
Ampicillin	0	0	29	100
Erythromycin	3	10.34	26	89.66
Vancomycin	27	93.10	2	6.90
Gentamicin	20	68.97	9	31.03
Amikacin	20	68.97	9	31.03
Cotrimoxazole	15	51.72	14	48.28
Cephalexin	0	0	29	100
Ceftrioxone	0	0	29	100
Ciprofloxacin	22	75.86	7	24.14
Ofloxacin	28	96.55	1	3.45
Linezolid	29	100	0	0
Doxycyclin	28	96.55	1	3.45

The antibiogram of MRSA showed sensitivity to Erythromycin (10.34%), Vancomycin (93.10%), Gentamicin (68.97%), Amikacin (68.97%), Cotrimoxazole (51.72%), Ciprofloxacin (75.86%), Ofloxacin (96.55%), Linezolid (100%), Doxycyclin (96.55%). Total resistance were noted in Ampicillin, Cephalexin and Ceftriaxone. Table 22

CHART - 17

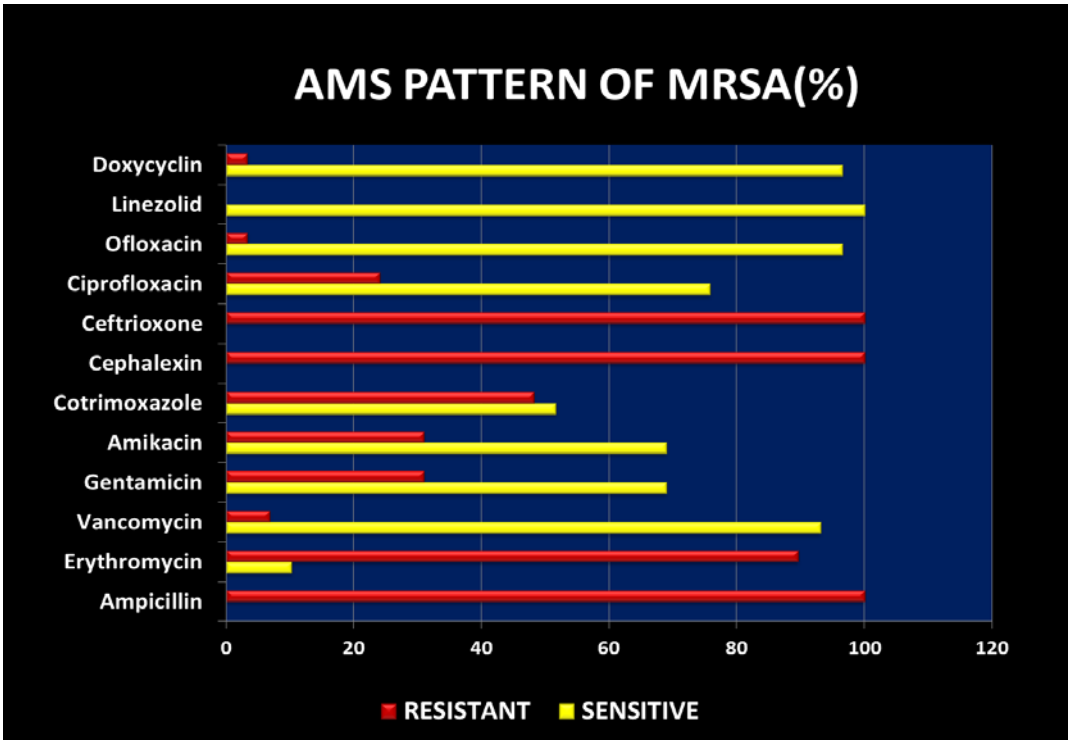


CHART - 18

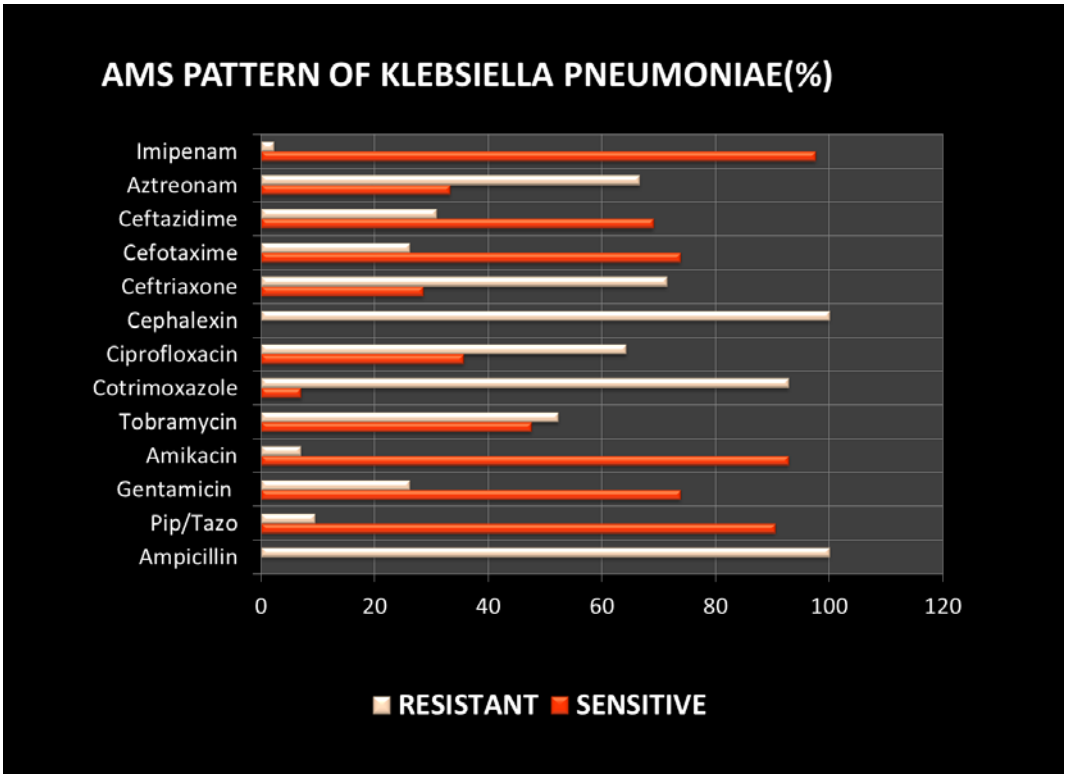


TABLE 23

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF KLEBSIELLA PNEUMONIAE

Organism	Klebsiella pneumonia (n = 42)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	42	100
Pip/Tazo	38	90.48	4	9.52
Gentamicin	31	73.81	11	26.19
Amikacin	39	92.86	3	7.14
Tobramycin	20	47.62	22	52.38
Cotrimoxazole	3	7.14	39	92.86
Ciprofloxacin	15	35.71	27	64.29
Cephalexin	0	0	42	100
Ceftriaxone	12	28.57	30	71.43
Cefotaxime	31	73.81	11	26.19
Ceftazidime	29	69.05	13	30.95
Aztreonam	14	33.33	28	66.67
Imipenam	41	97.62	1	2.38

The antibiogram of Klebsiella pneumonia showed sensitivity to Piperacillin/Tazobactum (90.48%), Gentamicin (73.81%), Amikacin(92.86%), Tobramycin(47.62%), Cotrimoxazole(7.14%), Ciprofloxacin (35.71%), Ceftriaxone (28.57%), Cefotaxime(73.81%), Ceftazidime (69.05%), Aztreonam (33.33%), and Imipenam (97.62%) among 42 isolates. The antibiogram showed total resistance to Ampicillin and Cephalexin. (Table 23)

TABLE 24

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI

Organism	Escherichia coli (n = 52)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	52	100
Pip/Tazo	42	80.77	10	19.23
Gentamicin	43	82.69	9	17.31
Amikacin	52	100	0	0
Tobramycin	34	65.38	18	34.62
Cotrimoxazole	11	21.15	41	78.85
Ciprofloxacin	29	55.77	23	44.23
Cephalexin	0	0	52	100
Ceftriaxone	16	30.77	36	69.23
Cefotaxime	29	55.77	23	44.23
Ceftazidime	32	61.54	20	38.46
Aztreonam	37	71.15	15	28.85
Imipenam	52	100	0	0

The antibiogram of Escherichia coli showed sensitivity to Piperacillin/Tazobactum (80.77%), Gentamicin (82.69%), Amikacin(100%), Tobramycin(65.38%), Cotrimoxazole(21.15%), Ciprofloxacin (55.77%), Ceftriaxone (30.77%), Cefotaxime(55.77%), Ceftazidime (61.54%), Aztreonam (71.15%), and Imipenam (100%) among 52 isolates. The antibiogram showed total resistance to Ampicillin and Cephalexin. (Table 24)

CHART - 19

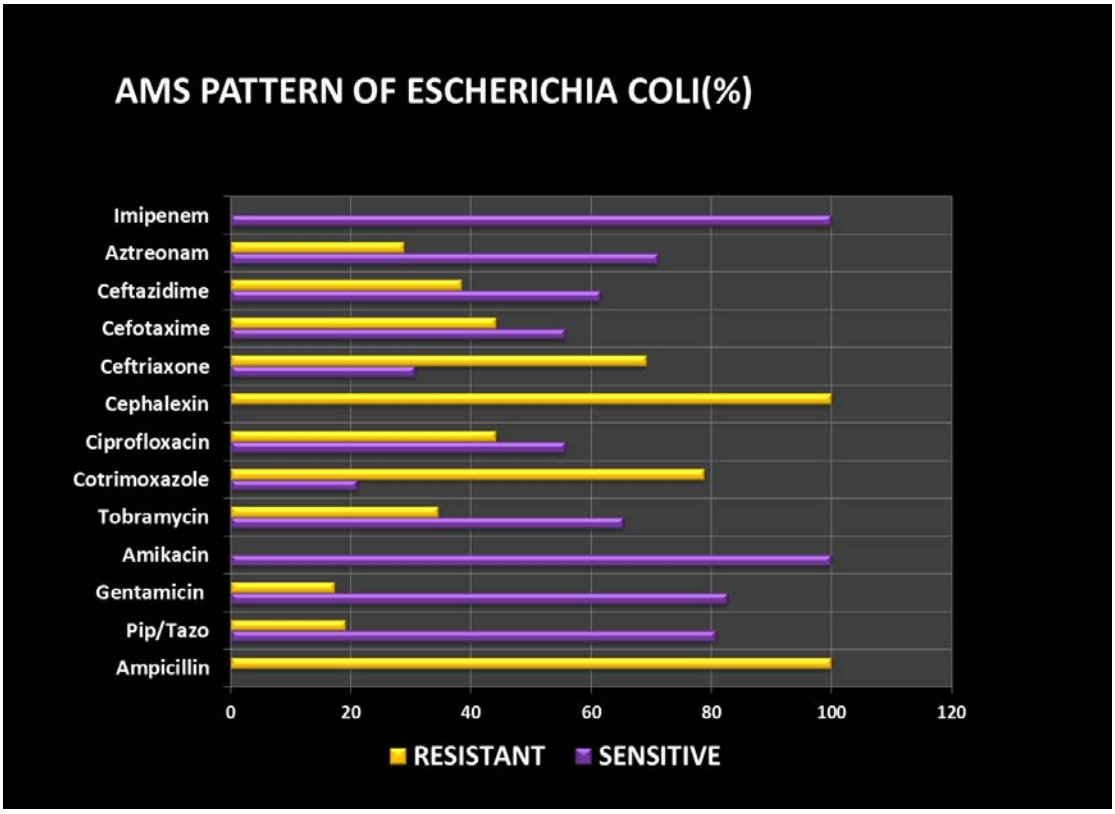


CHART 20

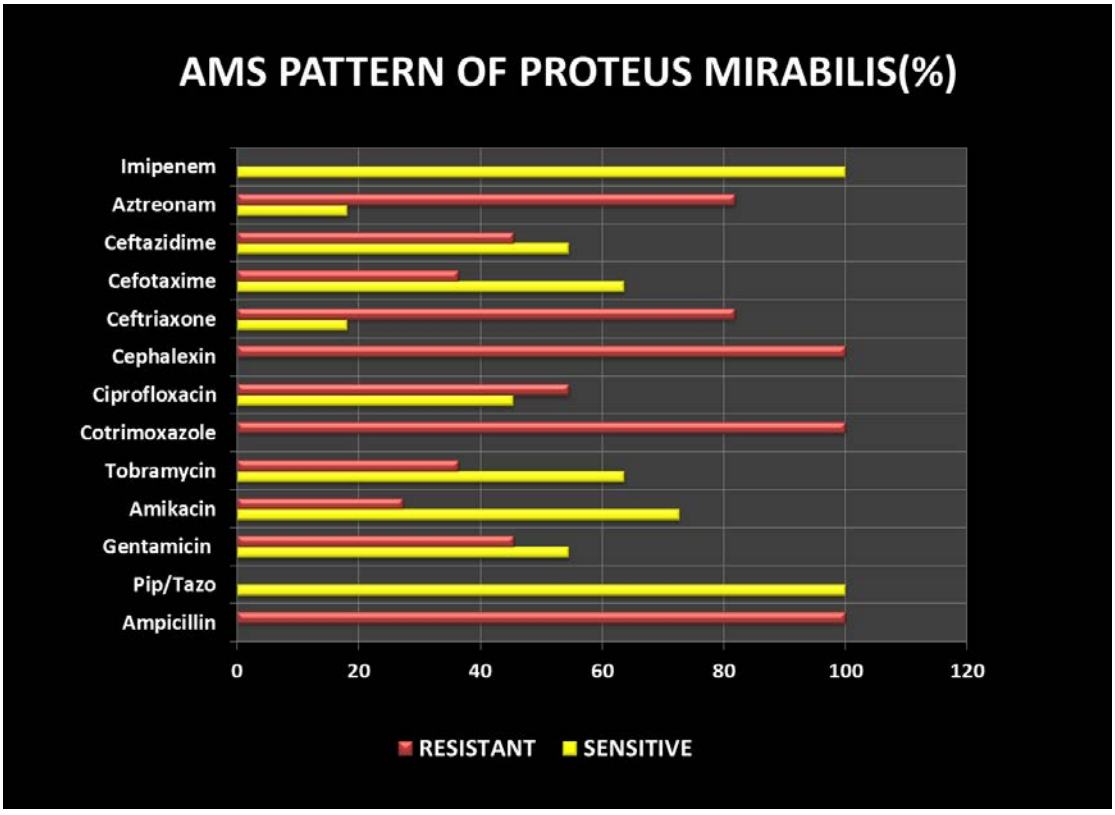


TABLE 25

ANTIMICROBIAL SENSITIVITY PATTERN OF PROTEUS MIRABILIS

Organism	Proteus mirabilis (n = 11)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	11	100
Pip/Tazo	11	100	0	0
Gentamicin	6	54.55	5	45.45
Amikacin	8	72.73	3	27.27
Tobramycin	7	63.64	4	36.36
Cotrimoxazole	0	0	11	100
Ciprofloxacin	5	45.45	6	54.55
Cephalexin	0	0	11	100
Ceftriaxone	2	18.18	9	81.82
Cefotaxime	7	63.64	4	36.36
Ceftazidime	6	54.55	5	45.45
Aztreonam	2	18.18	9	81.82
Imipenam	11	100	0	0

The antibiogram of Proteus mirabilis showed sensitivity to Piperacillin/Tazobactum (100%), Gentamicin (54.55%), Amikacin(72.73%), Tobramycin(63.64%), Ciprofloxacin (45.45%), Ceftriaxone (18.18%), Cefotaxime(63.64%), Ceftazidime (54.55%), Aztreonam (18.18%), and Imipenam (100%) among 11 isolates. The antibiogram showed total resistance to Ampicillin, Cotrimoxazole and Cephalexin. (Table 25)

TABLE 26

ANTIMICROBIAL SENSITIVITY PATTERN OF

CITROBACTER FREUNDII

Organism	Citrobacter freundii (n = 2)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	2	100
Pip/Tazo	2	100	0	0
Gentamicin	0	0	2	100
Amikacin	0	0	2	100
Tobramycin	1	50	1	50
Cotrimoxazole	0	0	2	100
Ciprofloxacin	0	0	2	100
Cephalexin	0	0	2	100
Ceftriaxone	0	0	2	100
Cefotaxime	2	100	0	0
Ceftazidime	2	100	0	0
Aztreonam	2	100	0	0
Imipenam	2	100	0	0

The antibiogram of Citrobacter freundii showed 100% sensitivity to Piperacillin/Tazobactum, Cefotaxime, Ceftazidime, Aztreonam and Imipenam and showed 50% sensitivity to Tobramycin among 2 isolates. Table 26

CHART - 21

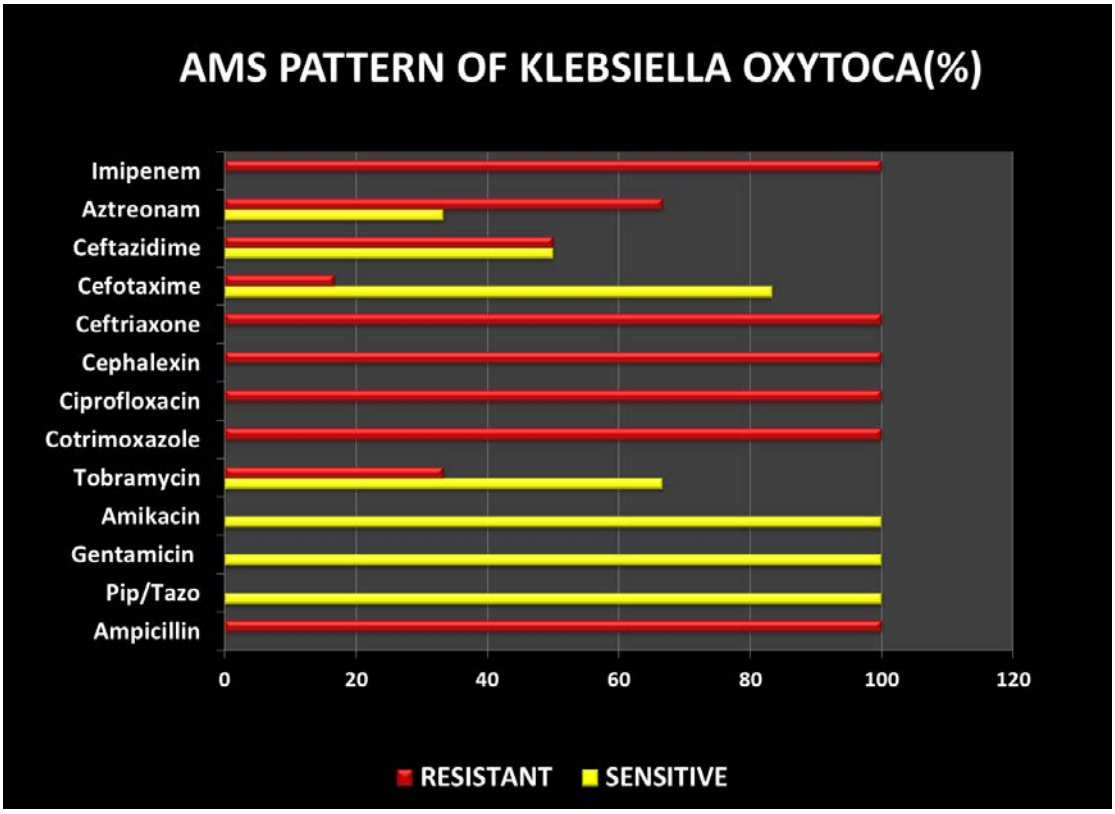


CHART - 22

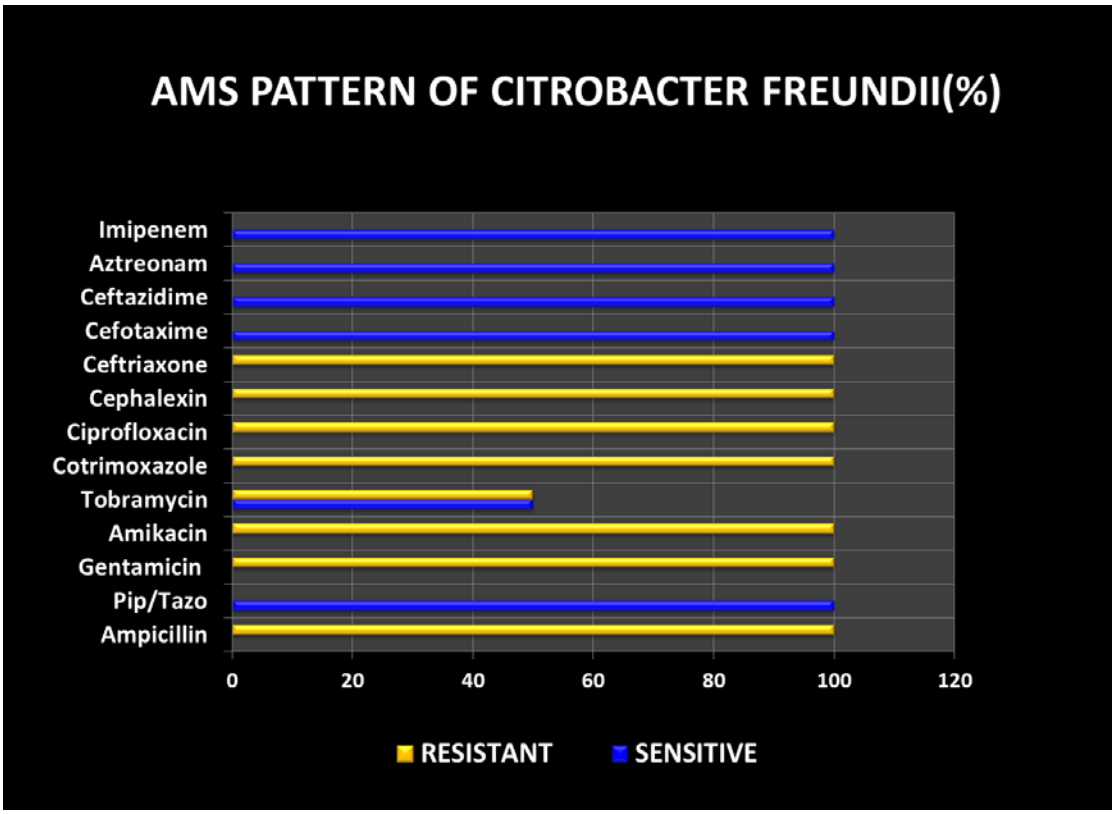


TABLE 27

ANTIMICROBIAL SENSITIVITY PATTERN OF

KLEBSIELLA OXYTOCA

Organism	Klebsiella oxytoca (n = 6)			
	Sensitive	Percenage	Resistant	Percentage
Ampicillin	0	0	6	100
Pip/Tazo	6	100	0	0
Gentamicin	6	100	0	0
Amikacin	6	100	0	0
Tobramycin	4	66.67	2	33.33
Cotrimoxazole	0	0	6	100
Ciprofloxacin	0	0	6	100
Cephalexin	0	0	6	100
Ceftriaxone	0	0	6	100
Cefotaxime	5	83.33	1	16.67
Ceftazidime	3	50	3	50
Aztreonam	2	33.33	4	66.67
Imipenam	6	0	0	100

The antibiogram of Klebsiella oxytoca showed 100% sensitivity to Piperacillin/Tazobactum, Gentamicin, Amikacin and Imipenam. It showed sensitivity to Tobramycin (66.67%), Cefotaxime (83.33%), Ceftazidime (50%), and Aztreonam (33.33%) among 6 isolates. (Table 27)

TABLE 28

ANTIMICROBIAL SENSITIVITY PATTERN OF ENTEROBACTER AEROGENES

Organism	Enterobacter aerogenes (n = 3)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	3	100
Pip/Tazo	3	100	0	0
Gentamicin	0	0	3	100
Amikacin	3	100	0	0
Tobramycin	1	33.33	2	66.67
Cotrimoxazole	0	0	3	100
Ciprofloxacin	0	0	3	100
Cephalexin	0	0	3	100
Ceftriaxone	0	0	3	100
Cefotaxime	3	100	0	0
Ceftazidime	1	33.33	2	66.67
Aztreonam	3	100	0	0
Imipenam	3	100	0	0

The antibiogram of Enterobacter aerogenes showed 100% sensitivity to Piperacillin/Tazobactum, Amikacin, Cefotaxime, Aztreonam and Imipenam. It showed 33.33% sensitivity to tobramycin and Ceftazidime among 3 isolates.(Table 28)

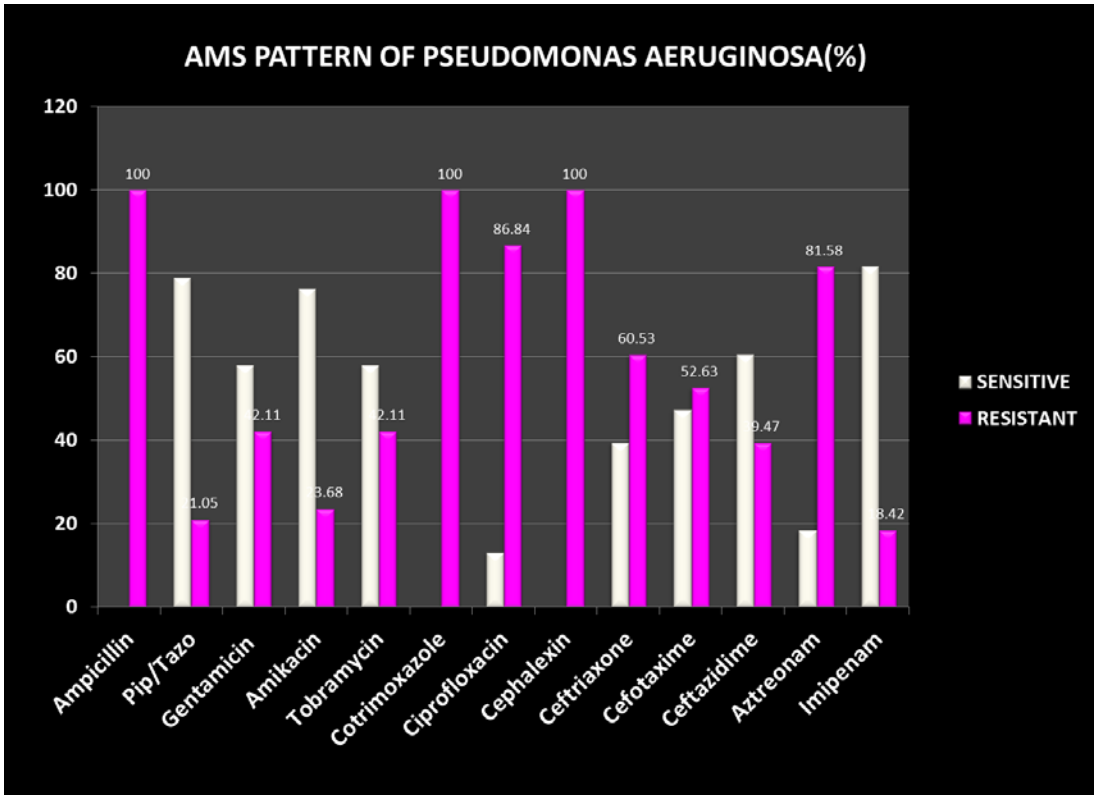
TABLE 29

ANTIMICROBIAL SENSITIVITY PATTERN OF PSEUDOMONAS AERUGINOSA

Organism	Pseudomonas aeruginosa (n = 38)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	38	100
Pip/Tazo	30	78.95	8	21.05
Gentamicin	22	57.89	16	42.11
Amikacin	29	76.32	9	23.68
Tobramycin	22	57.89	16	42.11
Cotrimoxazole	0	0	38	100
Ciprofloxacin	5	13.16	27	86.84
Cephalexin	0	0	38	100
Ceftriaxone	15	39.47	23	60.53
Cefotaxime	18	47.37	20	52.63
Ceftazidime	23	60.53	15	39.47
Aztreonam	7	18.42	31	81.58
Imipenam	31	81.58	7	18.42

The antibiogram of Pseudomonas aeruginosa showed sensitivity to Piperacillin/Tazobactum (78.95%), Gentamicin (57.89%), Amikacin (76.32%) Tobramycin (57.89%), Ciprofloxacin (13.16%),Ceftriaxone (39.47%),Cefotaxime (47.37%), Ceftazidime (60.53%), and Aztreonam (18.42%) and Imipenam (81.59%) among 38 isolates. Total resistance pattern was noted in Ampicillin, Cotrimoxazole and Cephalexin. (Table 29)

CHART - 23



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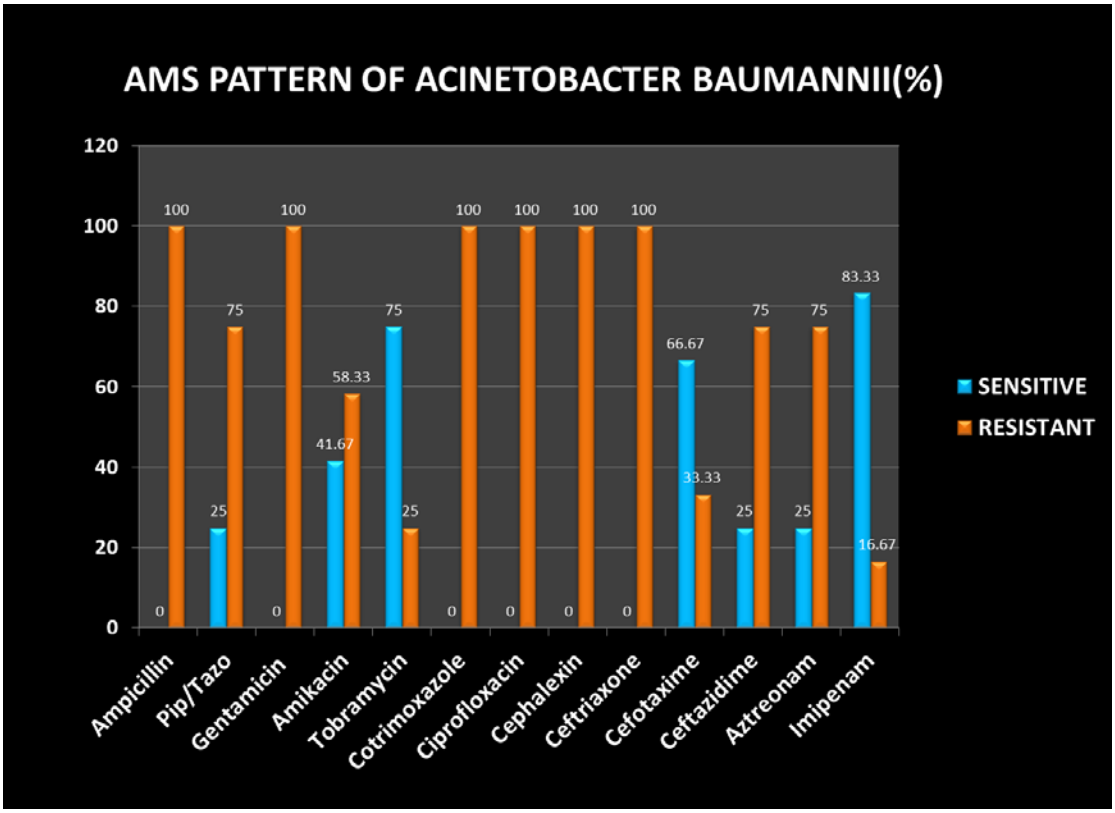


TABLE 30

ANTIMICROBIAL SENSITIVITY PATTERN OF ACINETOBACTER BAUMANNII

Organism	Acinetobacter baumanni (n = 12)			
	Sensitive	Percentage	Resistant	Percentage
Ampicillin	0	0	12	100
Pip/Tazo	3	25	9	75
Gentamicin	0	0	12	100
Amikacin	5	41.67	7	58.33
Tobramycin	9	75	3	25
Cotrimoxazole	0	0	12	100
Ciprofloxacin	0	0	12	100
Cephalexin	0	0	12	100
Ceftriaxone	0	0	12	100
Cefotaxime	8	66.67	4	33.33
Ceftazidime	3	25	9	75
Aztreonam	3	25	9	75
Imipenam	10	83.33	2	16.67

The antibiogram of Acinetobacter baumannii showed sensitivity to Piperacillin/Tazobactum (25%), Amikacin (41.67%) Tobramycin (75%), Cefotaxime (66.67%), Ceftazidime (25%), and Aztreonam (25%) and Imipenam (83.33%) among12 isolates. Total resistance pattern was noted in Ampicillin, Gentamicin, Cotrimoxazole ,Ciprofloxacin, Ceftriaxone and Cephalexin. Table 30.

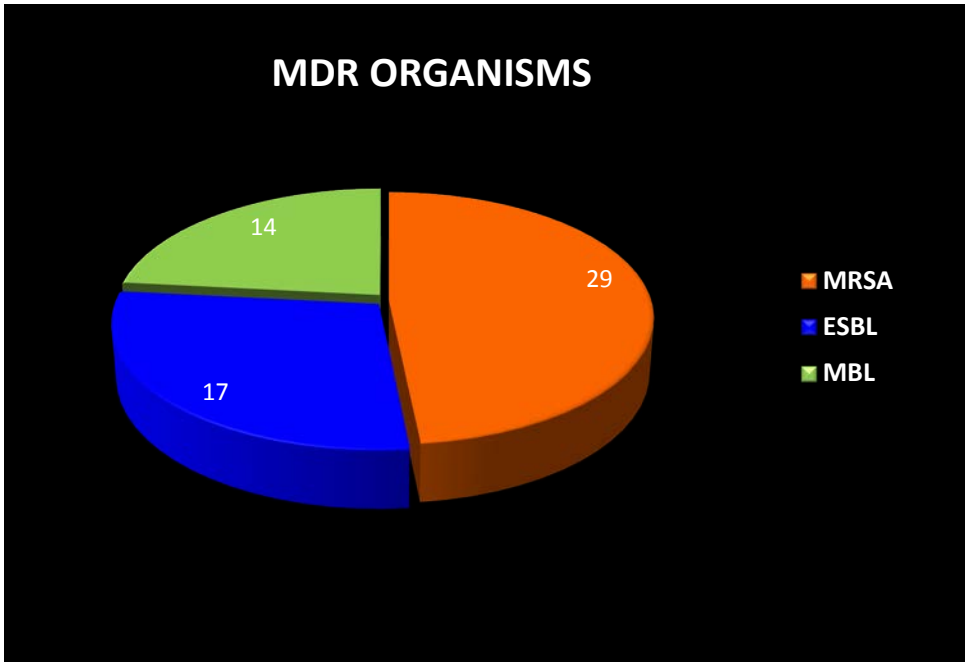
TABLE 31

DISTRIBUTION OF MDR AMONG ISOLATES

Organism	No of isolates	MDR		
		MRSA	ESBL	MBL
Staphylococcus aureus	125	29	-	-
Pseudo. aeruginosa	38	-	-	7
Acinet. baumanni	12	-	-	7
Klebsiella pneumoniae	42	-	8	-
Klebsiella oxytoca	06	-	3	-
Escherchia coli	52	-	6	-

The distribution of MDR organisms were studied from all the microorganisms isolated. Among 294 isolates, there was 29 MRSA, 17 ESBL producers and 14 MBL producers. Among 17 ESBL producers, Klebsiella pneumonia was 8, Klebsiella oxytoca was 3 and E.coli was 6. Among 14 MBL producers 7 were Pseudomonas aeruginosa and 7 were Acinetobacter baumanni.(Table 31)

CHART - 25



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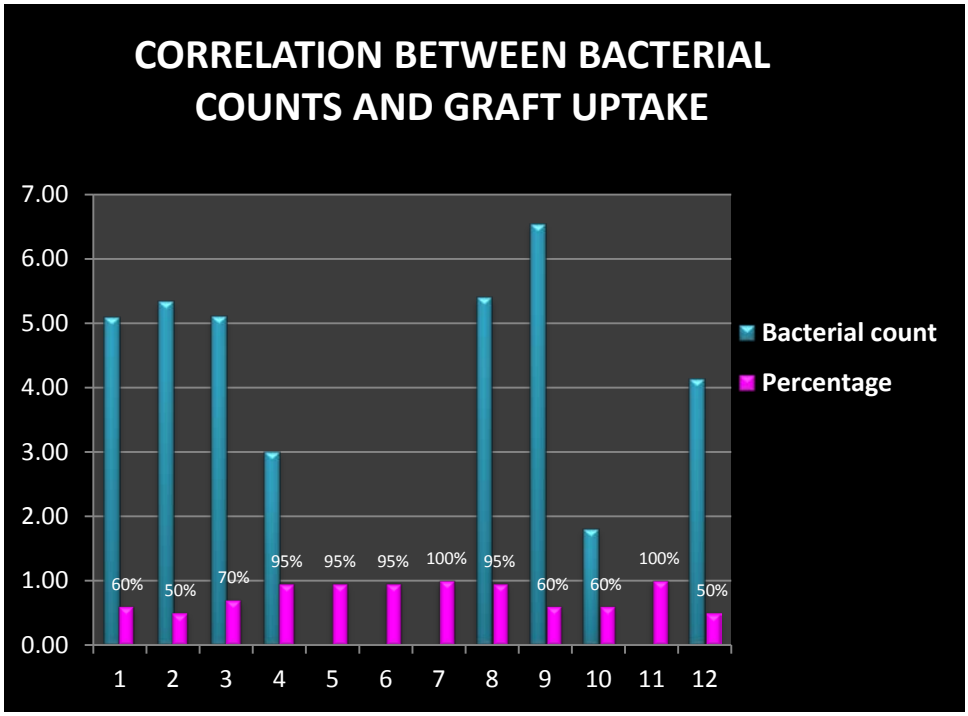


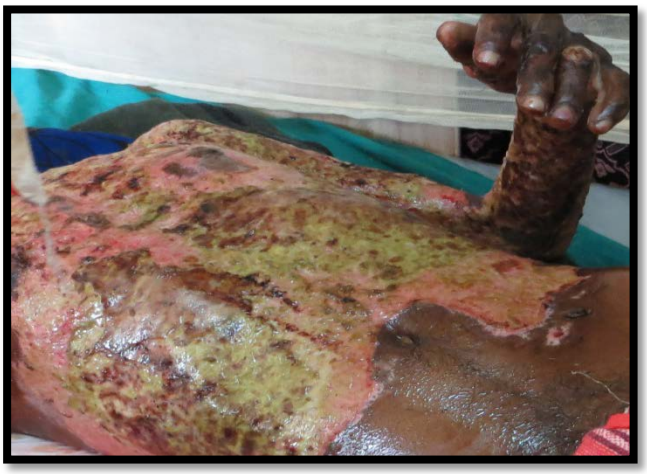
TABLE 32

CORRELATION BETWEEN BACTERIAL COUNTS AND CLINICAL OUTCOME

S. No	Age	Gender		Raw area %	Isolate	Bacterial count	Clinical intervention	Graft uptake
		M	F					
1	30		F	20	Staphylococcus aureus	5.09	SSG	60%
2	16	M		20	Staphylococcus aureus	5.34	SSG	50%
3	50	M		25	Pseudomonas aeruginosa	0.51	SSG	70%
4	20	M		20	Klebsiella pneumoniae	0.30	SSG	95%
5	48	M		30	-	NG	SSG	95%
6	19	M		10	Pseudomonas aeruginosa	Nil	SSG	70%
7	48	M		30	-	NG	SSG	100%
8	50		F	30	Klebsiella pneumoniae	0.54	SSG	95%
9	18		F	20	Staphylococcus aureus	6.54	SSG	60%
10	18		F	20	Pseudomonas aeruginosa	1.80	SSG	60%
11	22	M		30	-	NG	SSG	100%
12	60	M		15	Staphylococcus aureus	4.13	SSG	50%
Mean	33.5			22.5		2.18		
SD	16.48			6.57		2.67		
SEM	4.76			1.89		0.77		

RAW AREAS READY TO RECEIVE SKIN GRAFT

Out of 83 patients, this technique was employed in 12 burn patients whose raw areas were clinically ready for receiving skin graft . Tissue biopsy cultures and swab cultures were performed three days prior to the grafting procedures. Out of 12 patients, 8 patients were males and 4 patients were females. Their ages vary from 16 years to 60 years and were in the mean age group of 33.50 with SEM $\pm$ 4.76. Their total body surface areas burnt vary from 10% to 30% with a mean raw area ranging between 22.50 with SEM $\pm$ 1.89. Their bacterial counts ranged from 0.30 x 10^5 to 6.54 x 10^5 with a mean bacterial count of 2.18 with SEM $\pm$ 0.77.Staphylococcus aureus was isolated in 4 patients showed the highest bacterial counts. Pseudomonas aeruginosa was isolated in 3 patients and the bacterial counts ranged from 0.51 to 1.80. Klebsiella pneumoniae was isolated in 2 patients and their counts ranged from 0.30 to 0.54. Split skin graft was planned for all the patients. The “Graft Take″ for all the 12 patients were observed. Patients with bacterial counts ranging from 1.80 to 5.34 x 10^5 had a “graft take″ ranging from 50% to 60%. Patients with bacterial counts from 0.30 to 0.51 x 10^5 had a “graft take ” of 70% to 95%.Patients who were culture negative had a 95% to 100% “graft take”. (Table 32)



DISCUSSION

DISCUSSION

Infection in the burn wound continues to be the main cause of morbidity and mortality in patients who are admitted to hospital with major thermal burns.[104] Burns provide a suitable site for bacterial multiplication and more persistent richer sources of infection mainly because of larger area of involvement and longer duration of stay of the patients in the hospital.[105] Quantitative bacterial assay have been employed in this study to effectively monitor the burn wounds and to predict and prevent invasive burn wound sepsis at an early stage. Tissue biopsy samples have been taken and quantitative bacteriology was performed and also quantitative bacterial assay was compared with that of surface swabbing as it is the the chief modality of investigation in most of tertiary care centres.

In this study a total of 209 tissue biopsy samples and 83 surface swabs were collected from 83 burn wound patients admitted in burns ward. Tissue biopsy specimens were homogenized in a Lab Blender Stomacher 80, several dilutions were made and then processed simultaneously with that of surface swab cultures.

AGE AND GENDER PREVALENCE OF BURN WOUND INFECTIONS

Out of 83 cases, burn wound infections were most common in 16-30 age group (51.81%) followed by 31-40 age group (27.71%). Among the 83 patients, 45(54.22%) female and 38 (45.78%) were male affected by burn wound infections.

EXTENT OF BURN WOUND INFECTIONS

Out of 83 cases, 26 cases (31.33%) acquired 10-20% TBSA burns followed by 18 cases (21.69%) between 21-30% TBSA burns. Least number of cases were between 41-50% TBSA burns ie 5 cases (6.02%) . The burn wounds were studied according to the degree of burns depending upon the depth of skin involvement. Out of 83 cases, 35 cases had I degree burns (42.17%) followed by 26 cases who had III degree burns (31.32%).

PREVALENCE OF ISOLATES BY GRAM STAINING

Out 69 swab culture isolates, 45 samples (65.22%) were gram positive and 24 samples (34.78%) were gram negative. Out of 294 isolates obtained from the tissue biopsy culture, 128 samples were gram positive (43.54%) and 166 samples were gram negative (56.46%). The

surface swabbing showed gram positive predominance of organisms. Eventhough in tissue biopsy culture there was gram negative predominance, the most common isolate was Staphylococcus aureus. Various studies from routine burn wound culture surveillance at Boston displays a shift from gram negative to gram positive organisms and concluded that the most prevalent organisms are Methicillin sensitive Staphylococcus aureus.[106]McManus etal in his study stated that there was decrease in gram negative infections with improved isolation of burn patients.VG Bhat and SD Vasaikar substanstiated in their study[107] that the most common isolate was gram positive organism namely Staphylococcus aureus.Two Brazilian studies namely Macedo JLS et al[108] and Santucci SG et al[109] and one Malawi study namely Liwimbi et al[110] found Staphylococcus aureus as the predominant organism. On the other hand, two Nigerian studies namely Ozumba et al[111] and Kehinde et al[112]showed Klebsiella as the commonest organism. Two Indian studies namely Singh et al[113] and Kaushik R et al[114] showed Pseudomonas as the predominant organism.

SENSITIVITY OF TISSUE BIOPSY CULTURES AND SURFACE SWABBING CULTURES

Out of 83 surface swab cultures, 51 cases showed culture positive (61.45%) and 32 cases were culture negative (38.55%). Out of 209 tissue

biopsy samples, 199 cases showed culture positive (95.22%) and 10 cases were culture negative (4.78%). The tissue biopsy samples were taken on Days 1, 4 and 9. In the tissue biopsy specimens, out of 83 samples taken on Day 1, 74 cultures were positive (89.16%), out of 70 samples taken on Day 4, 69 cultures were positive (98.57%) and out of 56 samples taken on Day 9, 56 samples were culture positive (100%). It should be noted that 100% culture positives were present on Day 9 of tissue biopsy cultures. Moreover tissue biopsy cultures showed 95.22% culture positives whencompared with 61.45% culture positives in surface swabbing and cultures negatives were only 4.78% when compared to 38.55% of culture negatives in surface swabbing. This is a good evidence to show that tissue biopsy cultures are superior to surface swabbing.

Among the 294 isolates from the tissue biopsy specimens, 45 single isolates and 17 multiple isolates were found on samples taken on Day 1, 33 single isolates and 33 multiple isolates from sample taken on Day 4, and 20 single isolates and 36 multiple isolates from sample taken on Day 9. There was more prevalence of multiple isolates on Day 9 samples of tissue biopsy.

PREVALENCE OF ORGANISMS IN BURN WOUNDS

Surface swabbing showed Staphylococcus aureus (30.43%) was the commonest organism from the burn wounds followed by CoNS

(14.13%). The predominant organism isolated in the tissue biopsy culture was Staphylococcus aureus 32.65% followed by Escherichia coli 17.69% and Klebsiella pneumonia 14.29%. In surface swabbing, CoNS was reported in 14.13% and in many instances was reported as normal flora on clinical correlation. In case of tissue biopsy cultures only three isolates were reported as CoNS and were found to be sensitive to many drugs. More number of normal flora was also reported in surface swabbing such as Micrococcus, Diphtheroids and negative cultures were reported in 15.22% cases. But tissue biopsy cultures reflected the exact microorganism that existed in the sub eschar tissue at that period of time so that timely prediction and intervention may prevent mortality on a large basis.

Comparison of swab and tissue biopsy cultures also revealed a moderate concordance in detection of Staphylococcus aureus, low concordance in detection of Klebsiella oxytoca, Enterobacter aerogenes, and Proteus mirabilis.

QUANTIFICATION OF MICROORGANISMS

Out of the 294 isolates, Staphylococcus aureus (96) showed a mean bacterial count of 4.40 x 10^5, Pseudomonas aeruginosa (38) was 3.57 x 10^5, Klebsiella pneumonia (42) was 1.11 x 10^5, MRSA (29) was 4.78 x 10^5, Acinetobacter baumannii (12) was 1.74 x 10^5, Proteus mirabilis (11)

was 2.54 x 10^5, Citrobacter freundii (2) was 2.35 x 10^5, and Enterobacter aerogenes (3) was 2.40 x 10^5. Escherichia coli (52) and Klebsiella oxytoca (6) showed mean bacterial counts of 0.89 x 10^5 and 0.54 x 10^5 respectively which was less than 10^5 CFU/gm of tissue.

More than 10^5 organisms were found with Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, MRSA, Acinetobacter baumanni, Proteus mirabilis, Citrobacter freundii and Enterobacter aerogenes. Escherichia coli and Klebsiella oxytoca showed mean bacterial counts less than 10^5 CFU/gm of tissue and was considered as colonizers.

CORRELATION BETWEEN DEGREE AND BURN EXTENT AND BACTERIAL COUNTS

Bacterial counts were more than 10^5 regardless of extent and degree of burns but were 3.49 x 10^5 in 41-50% and 3.54 x 10^5 in 31-40% TBSA burns. It was 3.68 x 10^5 in II degree burns.

CHARACTERISTICS OF SURFACE AND BIOPSY CULTURE POSITIVE BURN PATIENTS

In the analysis, burn depth showed no significant association with the results of quantitative biopsy cultures. As for gender, there was significant association between the positive biopsy results and the female

gender ($p<0.05$). Age group showed a significant association with the results of biopsy cultures with the age group between 16 – 30 ($p<0.05$). There was significantly lower level of TBSA in patients with positive surface swab cultures compared with that biopsy cultures ($p<0.05$).

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Staphylococcus aureus showed sensitivity to Vancomycin (100%), Gentamicin (83.33%), Amikacin (92.71%), Cotrimoxazole (42.71%), Ciprofloxacin (86.46%), Ofloxacin (97.92%), Linezolid (96.87%) and Doxycyclin (92.71%). Total resistant patterns were noted in Cephelexin (100%) and relative resistant patterns were noted in Ampicillin (96.87%).The antibiogram of MRSA showed sensitivity to Erythromycin (10.34%), Vancomycin (93.10%), Gentamicin (68.97%), Amikacin (68.97%), Cotrimoxazole (51.72%), Ciprofloxacin (75.86%), Ofloxacin (96.55%), Linezolid (100%), Doxycyclin (96.55%). Total resistance were noted in Ampicillin, Cephalexin and Ceftrioxone. It should be noted that both Staphylococcus and MRSA showed good sensitivity pattern towards Cotrimoxazole and relative resistance was observed towards Cephalosporins and Penicillins.

Among the Enterobacteriaceae family, Klebsiella pneumonia was sensitive to Piperacillin/Tazobactum (90.48%), Gentamicin (73.81%), Amikacin(92.86%), Tobramycin(47.62%), Ciprofloxacin (35.71%),

Cefotaxime(73.81%), Ceftazidime (69.05%), Aztreonam (33.33%), and Imipenam (97.62%) among 42 isolates. The antibiogram of Escherichia coli showed sensitivity to Piperacillin/Tazobactum (80.77%), Gentamicin (82.69%), Amikacin(100%), Tobramycin(65.38%), Ciprofloxacin (55.77%), Ceftriaxone (30.77%), Cefotaxime(55.77%), Ceftazidime (61.54%), Aztreonam (71.15%), and Imipenam (100%) among 52 isolates. The antibiogram of Proteus mirabilis showed sensitivity to Piperacillin/Tazobactum (100%), Gentamicin (54.55%), Amikacin(72.73%), Tobramycin(63.64%), Ciprofloxacin (45.45%), Ceftriaxone (18.18%), Cefotaxime(63.64%), Ceftazidime (54.55%), and Imipenam (100%) among 11 isolates. The antibiogram of Citrobacter freundii showed 100% sensitivity to Piperacillin/Tazobactum, Cefotaxime, Ceftazidime, Aztreonam and Imipenem and showed 50% sensitivity to Tobramycin among 2 isolates. The antibiogram of Klebsiella oxytoca showed 100% sensitivity to Piperacillin/Tazobactum, Gentamicin, Amikacin and Imipenam. It showed sensitivity to Tobramycin (66.67%), Cefotaxime (83.33%), Ceftazidime (50%), and Aztreonam (33.33%) among 6 isolates. The antibiogram of Enterobacter aerogenes showed 100% sensitivity to Piperacillin/Tazobactum, Amikacin, Cefotaxime, Aztreonam and Imipenem. It showed 33.33% sensitivity to tobramycin and Ceftazidime among 3 isolates. In this study members of Enterobactericeae family was resistant to Ampicillin, most of

Cephalosporins and Cotrimoxazole. Proteus showed relative resistance to Aztreonam (81.82%).

The antibiogram of Pseudomonas aeruginosa showed sensitivity to Piperacillin/Tazobactum (78.95%), Gentamicin (57.89%), Amikacin (76.32%) Tobramycin (57.89%), Ceftriaxone (39.47%),Cefotaxime (47.37%), Ceftazidime (60.53%), and Imipenam (81.59%) among 38 isolates. Total resistance pattern was noted in Ampicillin, Cotrimoxazole and Cephalexin and relative resistance to ciprofloxacin and Aztreonam. The antibiogram of Acinetobacter baumannii showed sensitivity to Amikacin (41.67%) Tobramycin (75%), Cefotaxime (66.67%), and Imipenem (83.33%) among 12 isolates. Total resistance pattern was noted in Ampicillin, Gentamicin, Cotrimoxazole ,Ciprofloxacin, Ceftriaxone and Cephalexin and relative resistance toPiperacillin/Tazobactum, Ceftazidime and Aztreonam.

DISTRIBUTION OF MULTI DRUG RESISTANCE ORGANISMS

Among 294 isolates, there was 29 MRSA, 2 VRSA, 17 ESBL producers and 14 MBL producers. Among 17 ESBL producers, Klebsiella pneumonia was 8, Klebsiella oxytoca was 3 and E.coli was 6. Among 14 MBL producers 7 were Pseudomonas aeruginosa and 7 were Acinetobacter baumanni.

CORRELATION BETWEEN BACTERIAL COUNTS AND CLINICAL OUTCOME

Out of 83 patients, 12 patients who had raw areas which were ready to be grafted were chosen and studied prospectively. Tissue biopsy cultures were performed three days prior to the grafting procedures. Out of 12 patients, 8 patients were males and 4 patients were females. Their mean age vary from 16 years to 60 years and were in the mean age group of 33.50 with SEM $\pm$ 4.76. Their total body surface areas burnt vary from 10% to 30% with a mean raw area ranging between 22.50 with SEM $\pm$ 1.89. Their bacterial counts ranged from 0.30 x 10^5 to 6.54 x 10^5 with a mean bacterial count of 2.18 with SEM $\pm$ 0.77. The graft take was 95%-100% when the bacterial counts were less than 10^5 CFU/gm of tissue. But when the bacterial counts exceeded 10^5 CFU/gm of tissue, the efficacy of the clinical outcome decreased as was evidenced by the poor uptake of the graft, even when the clinical interventional procedures were carried out under good antibiotic coverage.

Steer and colleagues[88], in their study made a comparison between qualitative results and quantitative bacterial counts and concluded that the use of quantitative microbiology in burns is limited by the unreliability of

a single surface swab or biopsy sample to represent the whole burn wound.

Steer and coworkers[87] performed a clinical outcome study to determine the relationship between bacterial counts obtained by burn wound biopsy culture and surface swabs. Through their study they demonstrated that quantitative bacteriology by burn wound biopsy or surface swab sample does not aid the prediction of sepsis or graft loss.

Loebl and colleagues[82,85], demonstrated that the recovery of bacterial flora from the unexcised burn wound surface showed poor correlation with that from the tissue biopsy samples taken from deep sites beneath the eschar.

Freshwater and Su[79], also found that the results of quantitative burn wound cultures needed to be interpreted in conjunction with the clinical observations of burn wound infections in order to be a useful guide to the management of burn patients with large TBSA burns.

Tahlan and colleagues[83],in a study comparing surface swabs and burn wound biopsy cultures, found no difference in the types of microorganisms cultured from swabs versus those cultured from biopsies

Levine and colleagues[16], noted a linear numerical relationship between quantitative surface swab and biopsy sample counts of viable

bacteria from burn wounds, whereby counts of 10^5 bacteria per gram of biopsy sample were equated with counts of 10^6 bacteria obtained from surface swab samples.

McManus and colleagues [80] ,found that quantitative cultures of tissue biopsy samples provided a better determination of the predominant bacterial types present in the burn wound.

Herruzo-Cabrera and colleagues[89], showed that a semiquantitative surface swab method distinguished between wound contamination and infection, using 10^5 organisms/gram as a threshold for the definition of infection by biopsy.

Sjorberg and colleagues[86], reported that quantitative tissue biopsies gave a better prediction of sepsis than surface swabs but concluded that the amount of labour involved in collection and analysis of multiple biopsy samples limited the clinical relevance of this approach.

Bharadwaj and colleagues[26]also assessed the value of blood cultures in the diagnosis of burn wound sepsis compared to burn wound cultures by either swab or tissue biopsy. They concluded that blood cultures were found to be of only prognostic value in this study.Blood cultures have also been shown to be a late sign of invasive burn wound infection even when they are positive.[115]

Mohammad Ashraf Ganatra and colleagues,[100] in their study of method of quantitative bacterial count in burn wound, described the technique for conducting quantitative bacteriology in burn wounds and concluded that quantitative bacteriology is simple and easily adaptable and should be employed as the chief investigative tool for monitoring the bacterial count reaching the critical level of 10^5 per gram of tissue in every burn care unit.

STRENGTH OF THE STUDY

- Samples were collected, homogenized, quantitative bacterial assay done and processed by the same person, thereby eliminating the technical bias.
- Uniform and strict criteria were followed for case selection.
- Processing was done without delay in the shortest possible time.

LIMITATIONS OF THE STUDY

- Fungal isolates were not studied due to technical constraints.
- Anaerobic work up was not done due to the same reason
- Study population is limited to a single centre

SUGGESTIONS

Most common cause of mortality in burn patients are inhalation injury and systemic sepsis. All parameters should be taken care off to prevent impending sepsis.

- To prevent transmission of exogenous organisms to patients
- To control the transfer of endogenous organisms (normal flora) to sites at increased risk of infection
- To protect and support existing defences in patients with seriously impaired resistance
- To prevent dispersing of organisms into surrounding environment which is directly related to the percentage of burn injury
- Standard precautions and strict aseptic protocol in all burn centres
- Self-contained isolation rooms with separate burns bath facility
- Hand wash before and after each contact with the patient
- Appropriate garbs, aprons, masks and gloves
- Gloves changed after contamination with secretions and before contact with another patient
- Sterile practice when caring for an open wound and while preparing for performing sterile procedures
- Frequent decontamination of equipments, materials and surfaces in the burn unit

- Appropriate disinfection and sterilization procedures in the burn unit
- Appropriate disposal of waste encountered in the burn wounds
- Patient care items and equipments should be used according to CDC guidelines[116] Critical items should be sterile. Semi-critical items should undergo high level disinfection to kill all organisms except spores. Noncritical items which is an effective barrier to most organisms and represent little risk of transmission of infection.
- Routine surveillance of the burn patients is a must to allow early identification of organisms colonizing the wound
- To detect cross colonization quickly when it occurs to prevent further transmission
- To monitor the effectiveness of current wound treament strategies
- To guide perioperative or empiric antibiotic therapy
- Burn wound cultures by quantitative assays
- Bacterial counts more than 10^5 CFU/gm tissue is an indication of burn wound infection and alarming signal for excision of expedient eschar excision rather than starting antibiotics
- Aiming early eschar excision and skin grafting as it prevents burn wound colonization

- Surveillance of environmental pathogens to prevent noscomial infection
- Surveillance to prevent other complications like UTI, Pneumonia, and septicemia
- Monitoring of fungal pathogens especially in prolonged hospital stay.
- Prevention of injudicious use of antimicrobials as it leads to MDRO
- Recognition of resistant strains of organisms
- Always combination of topical and systemic antimicrobials should be attempted
- Each antimicrobial must be selected based on its effect and its specificity for the microbe present
- Decisions should be made on the basis of the culture and susceptibility data
- Drug selection should be according to the CLSI guidelines and should be separated into three categories such as primary use, selective use and reserve drugs
- The time, dosage, route of administration and duration of treatment should be in accordance with what is required in order to make the organism powerless

- Consideration should be given to local and systemic host resistant factors
- With these measures there would be reduction of mortality in burn wounds which at present is very high in India.

SUMMARY

The study was conducted at Thanjavur Medical College Hospital, Thanjavur over a period of June 2013 to July 2014. The study was conducted on 83 burn patients admitted here and samples consisting of both surface swab and tissue biopsy specimens were collected under strict aseptic procedures. The surface swabs were promptly processed. The tissue biopsy samples were homogenized in a homogenizer bag containing 1ml normal saline by Lab Blender Stomacher 80 for 30seconds.Then several dilutions were made out of the homogenate and inoculated in Blood agar plates and MacConkey agar plates. Colonies were counted and the effective bacterial counts obtained by the formula. Processing was done and the isolates were identified by appropriate biochemical reactions. Antimicrobial susceptibility testing was done for both the surface swabs and the tissue biopsy samples. Each isolate were screened for drug resistance. The study showed the following results.

- Out of 83 cases, 54.22% were females and 45.78% were males
- Out of 83 cases 51.81% of burn wounds were common in16-30 age group and 27.71% between 31-45 age group
- Out of 83 cases, 31.33% patients attained 10-20% TBSA burns and 21.69% attained 21-30% TBSA burns

- Out of 83 cases, 42.17% of patients acquired I degree burns, 31.32% III degree burns and 26.51% II degree burns
- Out of 69 isolates in surface swabbing cultures, 65.22% were gram positive and 34.78% were gram negative. Out of 294 isolates in tissue biopsy cultures, 43.54% were gram positive and 56.46% were gram negative.
- Out of 83 surface swab cultures, 61.45% were culture positive and 38.55% were culture negative. Out of 209 tissue biopsy cultures, 95.22% were culture positive and only 4.78% were culture negative.
- Out of 209 tissue biopsy cultures, 89.16% were culture positive on Day I, 98.57% were positive on Day 4 and 100% were culture positive on Day 9.
- Out of 294 isolates, 45 single isolates and 17 multiple isolates were present on Day1, 33 single isolates and 33 multiple isolate were present on day 4 and 20 single isolates and 36 multiple isolates were present on day 9.
- Out of 92 isolates in swab cultures, the predominant isolate was Staphylococcus aureus forming 30.43%. More CoNS, Micrococcus, Diphtheroids and culture negatives were reported in swab cultures.

- Out of 294 isolates in tissue biopsy isolates, Staphylococcus aureus was the predominant organism forming 32.65%. Less no of culture negatives and no normal flora was reported.
- On comparison of 92 surface swab cultures and 294 tissue biopsy cultures,there was moderate concordance for Staphylococcus aureus, low concordance for of Klebsiella oxytoca, Enterobacter aerogenes, and Proteus mirabilis.
- The mean bacterial counts were more than 10^5 organisms per gram of tissue for Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, MRSA, Acinetobacter baumanni, Proteus mirabilis, Citrobacter freundii abd Enterobacter aerogenes. The mean bacterial counts were less than 10^5 for E.coli and Klebsiella oxytoca and were considered to be colonizers.
- Correlation between burn wound extent and bacterial counts showed than in all percentage of TBSA burns the bacterial count exceeded 10^5. Similarly correlation between degree of burns and bacterial counts showed counts more than 10^5.
- There was significant association between positive biopsy results and the female gender ($p<0.05$), significant association between results of biopsy cultures and the age group between 16-30($p<0.05$)

and significant association between lower levels of TBSA in patients with positive surface swab cultures.($p<0.05$).

- Staphylococcus and MRSA were mostly sensitive to Vancomycin, Gentamicin, Amikacin, Ciprofloxacin,Cotrimoxazole,Ofloxacin ,Linezolid and Doxycyclin. They were resistant to Ampicillin, Cephalexin and Ceftriaxone.
- The Enteric group of organisms were mostly sensitive to Piperacillin/Tazobactum,Gentamicin, Amikacin,Tobramycin, Ciprofloxacin, Cefotaxime, Ceftazidime, Aztreonam, and Imipenam . They were resistant to Ampicillin, Ceftriaxone and Cephalexin. Only Proteus mirabilis showed relative resistance to Aztreonam.
- Pseudomonas aeruginosa was mostly sensitive to Piperacillin/Tazobactum,Genamicin,Amikacin,Tobramycin,Ceftazidime and Imipenam, less sensitive to Ceftriaxone, Cefotaxime,Ciprofloxacin and Aztreonam.They were resistant to Ampicillin, Cotrimoxazole and Cephalexin.
- Acinetobacter baumannii was sensitive to Tobramycin , Cefotaxime , and Imipenam. They were less sensitive to Piperacillin/Tazobactum, Amikacin, Ceftazidime and Aztreonam. They were resistant toAmpicillin, Gentamicin, Cotrimoxazole ,Ciprofloxacin, Ceftriaxone and Cephalexin.

- Distribution of MDR were found to be 29 MRSA, 17 ESBL producers and 14 MBL producers
- 12 burn patients whose raw areas were clinically ready for receiving skin graft were studied prospectively to know the clinical outcome. The graft take was 95%-100% when the bacterial counts were less than 10^5 CFU/gm of tissue. But when the bacterial counts exceeded 10^5 CFU/gm of tissue, the efficacy of the clinical outcome decreased as was evidenced by the poor uptake of the graft, even when the clinical interventional procedures were carried out under good antibiotic coverage.

CONCLUSION

CONCLUSION

Quantitative bacteriology of the burn wound should be the main investigative tool for monitoring the burn wound infections in order to prevent bacterial count reaching the critical level of 10^5 bacteria per gram of tissue. Staphylococcus aureus was the predominant organism isolated with bacterial counts more than 10^5 per gram of tissue. Comparison of the tissue biopsy with that of surface swabbing proved that quantitative bacteriology reflected the exact microbial load in the subeschar tissue with bacterial counts exceeding 10^5 bacteria per gram of tissue for most of the organisms, while surface swabbing did not reflect the exact pathogen status. In this study, quantitative bacteriology proved very useful in surgical intervention by reduction of the microbial load by debridement and skin grafting, provided that the graft bed contains less than 10^5 bacteria per gram of tissue. When colony counts were less than 10^2, graft survival was greater than 90%, but when colony counts were greater than 10^5, only a 60% graft survival rate was observed. On the basis of quantitative bacteriology, an effective antimicrobial battery of drugs has been formulated, specific to this hospital and community environment. Each antimicrobials can be selected based on its effect and specificity for **that microbe** present, as quantitative bacteriology eliminates the contaminants and commensals and focuses on the

identification of the specific organism. Serial quantitative bacterial counts helps to alert the clinician about the evolution of colonizers to critical levels and hence decide about timely surgical intervention such as escharectomy, consequently preventing the onset of septicaemia.Distribution of MDRorganisms from the burn wound isolates were identified and an effective antibiotic policy has been formulated. Judicious use of third generation Cephalosporins with appropriate MIC for Ceftazidime, Ceftriaxone,Cefotaxime will take care of the MDR organisms. Increase degrees of susceptibility to cotrimoxazole among the gram positive isolates including MRSA and some gram negative isolates suggest adequate "**antibiotic holidays**" can be allowed to those drugs which suffer extensive resistance like Ampicillin, Erythromycin and Cephalexin for a short course of time. This study has proved that quantitative cultures obtained on a routine basis will monitor the progress of bacterial colonization, provide guidance in empirical antibiotic therapy and also allow prompt intervention against bacterial invasion. Here, the method described in this study is simple and easily adaptable. So it is recommended that it should be employed in every public and private burn care units all over India.

ANNEXURES

INFORMED CONSENT

I have been informed about the study of Burn wound infections. I totally agree to participate in this study, as I realize the importance of the study. I am also aware that I can withdraw from the study whenever I want.

Date: Signature of the patient

Department:

BURNS WOUND CASE HISTORY

Name : IP No/OP No :
Age/Sex : Ward :

Address : Central Lab No :

Occupation : Micro No :

Diagnosis : Date of sample collection:

Chief Complaints:

Nature of injury :

Onset of injury :

Total area of burns:

Breathlessness :

Fever :

Altered consciousness:

Urine output:

Past History:

Treatment History:

Diabetes:

Hypertension:

Personal History:

Smoker : Alcoholic: Menstrual:

Clinical diagnosis and treatment plan:

WORKSHEET

Specimen	:	Pus,aspirate
Method of collection	:	Deep swabbing, Aspiration, Curetting
I. Macroscopic Examination	:	Consistency, Presence of blood, Colour, Odour
II. Microscopic Examination	:	Direst gram staining
III. Culture		
Nutrient agar	:	
MacConkey agar	:	
Blood agar	:	
Gram staining	:	
Motility	:	
IV. Biochemical Reactions		
Catalase	:	
Oxidase	:	
Sugar fermentation tests	:	
IMViC	:	
Urease	:	
TSI	:	
LAO	:	Special Tests:
Coagulase	:	
Micro organism isolated	:	
V. Anti Microbial Susceptibility test	:	Antibiogram on MHA by Kirby Bauyer method

ANTIBIOGRAM

S.No	DRUG	A	B	C	Remarks
1.	Ampicillin				
2.	Amoxycillin				
3.	Amoxyclav				
4.	Oxacillin				
5.	Erythromycin				
6.	Azithromycin				
7.	Cotimoxazole				
8.	Doxycycline				
9.	Amikacin				
10.	Gentamicin				
11.	Ciprofloxacin				
12.	Ofloxacin				
13.	Cephelexin				
14.	Cefixime				
15.	Cefotaxime				
16.	Ceftrioxone				
17.	Ceftazidime				
18.	Ceftazidime+clav				
19.	Cefipime				
20.	Piperacillin+Tazo				
21.	Aztreonam				
22.	Imipenam				
23.	Vancomycin				

QUANTITATIVE BACTERIALASSAY

1/10	1/100
1/1000	1/10000

Day 1	Weight of tissue	
	Isolate	Colony count
1		
2		

1/10	1/100
1/1000	1/10000

Day 2	Weight of tissue	
	Isolate	Colony count
1		
2		

1/10	1/100
1/1000	1/10000

Day 9	Weight of tissue	
	Isolate	Colony count
1		
2		

ANTIBIOGRAM

S.No	Drugs	A	B	C	Remarks
1.	Ampicillin				
2.	Amoxycillin				
3.	Amoxyclav				
4.	Linezolid				
5.	Erythromycin				
6.	Tobramycin				
7.	Cotimoxazole				
8.	Doxycycline				
9.	Amikacin				
10.	Gentamicin				
11.	Ciprofloxacin				
12.	Ofloxacin				
13.	Cephelexin				
14.	Cefixime				
15.	Cefotaxime				
16.	Ceftrioxone				
17.	Ceftazidime				
18.	Ceftazidime+clav				
19.	Cefipime				
20.	Piperacillin+Tazo				
21.	Aztreonam				
22.	Imipenam				
23.	Vancomycin				

MEDIA PREPARATION

NUTRIENT AGAR:

2.8 gm of dehydrated nutrient agar powder is taken and 100 ml of distill water is added. It was boiled to dissolve it completely. pH is adjusted to 7.4. Autoclaved at 121°C for 15 min and plated.

MACCONKEY AGAR:

5.5 gm dehydrated MacConkey agar is taken and 100 ml distill water is added. Then it is heated to dissolve completely. pH adjusted to 7.4. Then autoclaved at 121 ° C for 15 min.

BLOOD AGAR:

8 ml of human blood is added to the prepared nutrient agar. Mix thoroughly .then autoclaved at 121 ° C for 15 min and plated.

MUELLER-HINTON AGAR:

3.8 gm of dehydrated media is taken and 100 ml of distilled water is added. Then it is heated to dissolve completely. pH is adjusted to 7.3.Them autoclaved at 121° C for 15 min. Then plated.

GRAM STAIN:

Methyl violet:

Methyl violet – 1.5 gm

Distilled water – 100 ml

Grams iodine

Potassium iodide – 2 gm

Iodine - 2 gm

Water – 100 ml

DILUTE CARBOL FUCHSCIN:

Strong carbolfuchscin:

Basic fuchscin – 0.5 gm

Phenol crystal – 2.5 gm

Alcohol – 10 ml

Water - 90 ml

Take 1 ml of strong carbolfuchscin and add 19 ml of distill water.

INDOLE TEST:

Peptone water:

Peptone - 5gm

Sodium chloride – 2.5 gm

Water – 500 ml

Autoclave at 121 ° C for 15 minutes

METHYL RED TEST/VOGES PROSKAUER TEST:

MR-VP broth :

1.7 gm of glucose phosphate broth is taken and 100 ml distilled water is added and it heated to dissolve completely. pH adjusted to 6.9. Then autoclaved at 121 ° C for 15 min. Then dispensed in separate tubes.

MR reagent:

Methyl red - 0.1 gm

95 % Ethyl alcohol – 300 ml

VP reagent 1:

α- naphthol – 5 gm

Absolute ethyl alcohol – 100 ml

VP reagent 2:

Potassium hydroxide – 40 gm

Distilled water – 100 ml

CITRATE UTILIZATION TEST

2.428 gm of dehydrated citrate powder is taken in a conical flask and 100 ml of distilled water is added. It was boiled till it dissolved completely. pH adjusted to 6.8. Then autoclaved at 121° C for 15 min.

UREASE TEST:

2.401 gm of dehydrated urea agar base is taken and 95 ml of distilled water is added. It was boiled to dissolve it completely. pH adjusted to 6.8. Then autoclaved at 121° C for 15 min and then cooled to 50° C. 5 ml of 40% of urease solution is added.

TRIPLE SUGAR IRON AGAR:

6.452 gm of dehydrated medium is taken and mixed with 100 ml of distill water. Then it is heated to dissolve. pH is adjusted to 7.4.Then autoclaved at

121˚C for 15 min. Then it is dispensed in test tubes in such a way to form a slant and butt.

NITRATE REDUCTION TEST:

Nitrate broth:

0.9 gm of dehydrated medium is taken and mixed with 100 ml of distilled water. Heat to dissolve completely.pH is adjusted to 7. Then autoclave at 121° C for 15 min. Then it is dispensed in test tubes.

Nitrate reagent:

α- Naphthylamine – 0.5 gm

30% Acetic acid – 100 ml

OF TEST:

1.935 gm of dehydrated Hugh-Leifson's OF medium is taken and 100 ml of distilled water is added and boiled it to dissolve completely. pH adjusted to 6.8 and then dispensed in separate tubes. Autoclaved at 121° c for 15 min.

LAO DECARBOXYLATION TEST:

1.052 gm of dehydrated decarboxylase base is taken and 100 ml of distilled water is added. 10 gm of Lysine, arginine and ornithine are taken separately and is added and boiled to dissolve it completely. pH adjusted to 6. Dispensed in 5 ml amounts in separate tubes and then autoclaved.

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